Recommendations for Eradication and Control of Non-indigenous, Colonial, Ascidian Tunicates in Newfoundland Harbours

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RECOMMENDATIONS FOR ERADICATION AND CONTROL OF NON-INDIGENOUS, COLONIAL, ASCIDIAN TUNICATES IN NEWFOUNDLAND HARBOURS

by

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TABLE OF CONTENTS

LIST OF FIGURES VI

ABSTRACT VII

RÉSUMÉ VII

DISCLAIMER VIII

PREFACE: EXECUTIVE SUMMARY IX

ACKNOWLEDGEMENTS XI

1.0 INTRODUCTION 1

2.0 DESCRIPTION OF *BOTRYLLUS SCHLOSSERI* AND *BOTRYLLOIDES VIOLACEUS* 2

2.1 Taxonomy and identification 2

2.1.1 General biology 2

2.1.2 Origin and morphology 3

2.1.3 Genetics 5

2.2 Distribution 6

2.2.1 Global 6

2.2.2 Regional 6

2.2.3 Local 7

2.3 Habitat and growth 9

2.3.1 Depth range 9

2.3.2 Preferred substrates 9

2.3.3 Asexual growth 9

2.3.4 Seasonal pattern of growth 10

2.4 Factors affecting growth and reproduction 12

2.4.1 Temperature control of the life cycle 12

2.4.2 Salinity 13

2.4.3 Predators 13

2.4.4 Light 14

2.5 Reproduction and dispersal 14

2.5.1 Asexual reproduction 14

2.5.2 Sexual reproduction 15

2.5.3 Natural dispersal 16

2.5.4 Anthropogenic dispersal 16

3.0 RISK ANALYSIS OF INVASION 20

3.1 How long have *Botryllus schlosseri* and *Botrylloides violaceus* been in Newfoundland? 20

3.2 What is the likely invasion habitat in Newfoundland? 21

3.3 What is the present extent of *Botrylloides violaceus* in Belleoram Harbour? 21

3.4 What is the present extent of *Botryllus schlosseri* in Newfoundland? 22

3.5 Will *Botryllus schlosseri* and *Botrylloides violaceus* die out naturally? 22

3.6 What is the possibility for regional spread around the island? 22

3.7 How likely is the invasion of mussel farms? 22
3.8 How likely is the invasion of salmon farms? 23
3.9 What has been the economic impact elsewhere in the region, and in Canada? 23
4.0 FEASIBILITY OF ERADICATION 24
4.1 Importance of early detection and local science 24
4.1.1 Surveys 24
4.1.2 Species-specific genetic markers to detect fragments or larvae in plankton tows 25
4.1.3 Site-specific information on the seasonal cycles of growth, reproduction, larval settlement, and temperature 26
4.2 Methods 26
4.2.1 Global and regional 26
4.2.2 Local 28
4.2.3 Success rate and factors global and regional 28
4.2.4 Success rate and factors at local scales 32
4.2.5 Probability of successful eradication 34
4.2.6 Risks 34
5.0 FEASIBILITY OF CONTROL 35
5.1 The importance of monitoring 35
5.1.1 Rapid Assessment Surveys (RAS) 35
5.1.2 Gene probes 35
5.1.3 Passive surveys 35
5.2 Importance of public vigilance 35
5.3 Vector control 35
5.4 Importance of public compliance and the lack of political will 36
5.5 Risks 36
6.0 RESPONSE OPTIONS 37
6.1 Do nothing 37
6.2 Live with it 37
6.3 Stakeholders take the responsibility 38
6.4 Map, monitor, and inform 38
6.5 Vector control: voluntary 38
6.6 Vector control: mandatory 38
6.7 Eradicate 39
7.0 RECOMMENDATIONS 39
7.1 Surveillance 41
7.1.1 Passive survey 41
7.1.2 Rapid assessment surveys 42
7.1.3 Genetic assays 42
7.2 Public education and stakeholder consultation 42
7.3 Prevention 43

7.4 Eradication 44
7.4.1 Timing 44
7.4.2 Method 44
7.5 Control 45
7.6 Minimize risks of unintended consequences 45
7.7 Future research 46
7.7.1 Sediment suffocation and air bubbles 46
7.7.2 Ultrasound 46
7.7.3 Hull Cleaning 47
REFERENCES 48

LIST OF FIGURES

Figure 1. Global occurrence of *Botryllus schlosseri* as reflected by information from several databases 3

Figure 2. Global occurrence of *Botrylloides violaceus* as reflected by information from several databases 4

Figure 3. A colony of *Botryllus schlosseri*. Note the stellate arrangement of individual zooids into separate systems making up the colony 4

Figure 4. Two colonies of *Botrylloides violaceus* of differing colour morphotype 5

Figure 5. Map of the island of Newfoundland showing those locations at which *Botryllus schlosseri* and *Botrylloides violaceus* have been found as of April 2013 8

Figure 6. The annual cycle of asexual growth, sexual reproduction, and larval recruitment of *Botryllus schlosseri* in Arnold's Cove, Newfoundland 11

Figure 7. Common naturally occurring substrates for the attachment of *Botryllus schlosseri* and *Botrylloides violaceus* 17

Figure 8. Hull fouling by *Botryllus schlosseri* and *Botrylloides violaceus* 19

Figure 9. Map of fishing vessel traffic between the island of Newfoundland and the Maritime Provinces of Canada 20

Figure 10. Common anthropogenic substrates fouled by *Botryllus schlosseri* and *Botrylloides violaceus* 21

Figure 11. Encapsulation of a fixed wharf piling in Belleoram, Newfoundland, with sheets of plastic 31

Figure 12. Plastic sheeting on wharf pilings covered by colonies of *Botrylloides violaceus* in Belleoram, Newfoundland 33

Figure 13. Colonies of *Botrylloides violaceus* of two colour morphotypes living in small crevices deep within the fixed wharf substructure in Belleoram, Newfoundland 33

ABSTRACT

Deibel, D., McKenzie, C.H., Rise, M.L., Thompson, R.J., Lowen, J.B., Ma, K.C.K., Applin, G., O'Donnell, R., Wells, T., Hall, J.R., Sargent, P. and Pilgrim, B.B. 2014. Recommendations for eradication and control of non-indigenous, colonial, ascidian tunicates in Newfoundland harbours. Can. Manuscr. Rep. Fish. Aquat. Sci. 3039: xi + 60 p.

Two non-indigenous, colonial, ascidian species, *Botryllus schlosseri* and *Botrylloides violaceus*, were discovered along the south coast of Newfoundland in 2006-2007. *B. schlosseri* was found in several harbours, while *B. violaceus* has been found in one harbour thus far. These species have been reported in high, invasive abundances in many parts of the world, including other locations in Canada. They can have severe impacts on the marine environment, including displacing native benthic species, and threatening the economic sustainability of bivalve aquaculture farms. In this manuscript, we review the biology of these two species, their genetics and population ecology, and make recommendations for their eradication and control in Newfoundland harbours. This report is based on four years of research we have done on these non-indigenous ascidians in Belleoram and Arnold's Cove, Newfoundland.

RÉSUMÉ

Deibel, D., McKenzie, C.H., Rise, M.L., Thompson, R.J., Lowen, J.B., Ma, K.C.K., Applin, G., O'Donnell, R., Wells, T., Hall, J.R., Sargent, P. and Pilgrim, B.B. 2014. Recommendations for eradication and control of non-indigenous, colonial, ascidian tunicates in Newfoundland harbours. Can. Manuscr. Rep. Fish. Aquat. Sci. 3039 : xi + 60 p.

Deux espèces d'ascidies coloniales non indigènes, *Botryllus schlosseri* et *Botrylloides violaceus*, ont été découvertes le long de la côte sud de Terre-Neuve en 2006-07. Jusqu'à présent, la présence de *B. schlosseri* a été notée dans plusieurs ports, alors que celle de *B. violaceus* n'a été détectée que dans un seul. Ces espèces invasives ont été rapportées en grande abondance dans plusieurs parties du monde, incluant d'autrés regions du Canada. Leur impact sur l'environnement marin peut être sévère et inclut le déplacement d'espèces benthiques indigènes ainsi que la mise en péril de la viabilité économique des fermes aquacoles de bivalves. Dans ce manuscrit, nous passons en revue la biologie de ces deux espèces d'ascidies non indigènes, leur génétique et l'écologie de leurs populations et présentons nos recommandations pour leur éradication et leur contrôle dans les ports de Terre-Neuve. Ce rapport est basé sur quatre ans de recherches, conduites à Belleoram et à Arnold's Cove, Terre-Neuve.

DISCLAIMER

The authors of this report have made every effort to ensure that the information it contains is accurate; however, such information is provided on an 'as is' basis and is not intended to be a substitute for professional advice. The authors, collectively and individually, do not guarantee or warrant the quality, accuracy, completeness, timeliness, appropriateness or suitability of such information and are not responsible or liable for any errors, omissions, interpretations or opinions which are contained herein, nor for the consequences of any decisions taken that are based in whole or in part upon this report, nor for any use of this report for any purpose. The authors accept no liability in contract, tort, negligence, equity or otherwise for any loss, damage, injury, or expense, whether direct, indirect or consequential, arising from decisions or actions taken based in whole or in part upon or using this report. Any view, opinion, or recommendation expressed by the authors does not necessarily represent the official view of Memorial University of Newfoundland, the Province of Newfoundland and Labrador (Department of Fisheries and Aquaculture), the Government of Canada (Department of Fisheries and Oceans, and the Natural Sciences and Engineering Research Council), or the Newfoundland Aquaculture Industry Association (NAIA).

PREFACE: EXECUTIVE SUMMARY

The Golden Star Tunicate, *Botryllus schlosseri*, was first reported in Newfoundland in 1951. We have now found it in 23 harbours, primarily in Placentia and Fortune bays, on the south coast of Newfoundland. *B. schlosseri* is a widely distributed ascidian in the northern hemisphere, thought to have originated from the eastern Mediterranean Sea. Genetic research that we have done indicates that the probable origins of Newfoundland populations of *B. schlosseri* are ports on the Mediterranean Sea and Atlantic Ocean coasts of France and Spain. In Newfoundland, *B. schlosseri* is most commonly found fouling wharf pilings, ballast rocks and the hulls of coastal vessels. This ascidian has not been reported as a pest on any bivalve aquaculture farm in Newfoundland.

The Violet Tunicate, *Botrylloides violaceus*, was first reported in Newfoundland by our research team in 2006. As of March 2013, we have found *B. violaceus* only in Belleoram harbour, Fortune Bay. This non-indigenous ascidian is thought to have originated from the western Pacific Ocean, perhaps Japan. *B. violaceus* is found in Newfoundland fouling similar substrates as *B. schlosseri*, and has also not been reported as a pest for bivalve aquaculture in the province.

Both of these colonial ascidians are difficult if not impossible to eradicate. They can inhabit small spaces deep within wharf substructures. They also can cling tenaciously to the hulls of vessels due to their low, encrusting profile. Both species have high rates of asexual growth and sexual reproduction in Newfoundland waters. *Botryllus schlosseri* also has high rates of survival over the long, cold Newfoundland winter. These overwintering survivors become mature and release larvae the following summer. Thus, Newfoundland populations are self-sustaining.

We have tested several methods for eradication of *Botryllus schlosseri* and *Botrylloides violaceus* in Newfoundland, including wrapping wharf pilings and vessels in plastic sheets (i.e., encapsulation), removing floating docks from the water during winter, and introducing sea urchins as predators. None of these methods has been effective in eradicating these ascidians for more than one year. Removing floating docks during winter has been most effective, while encapsulation has also reduced the abundance of these species within the first year of the trial. Sea urchins were not effective.

We recommend a deliberate, hierarchical approach to the control of *Botryllus schlosseri* and *Botrylloides violaceus* in Newfoundland harbours. Since they are both present in Newfoundland, the primary objective should be to prevent further spread to harbours and aquaculture farms. This means that coastal vessels sailing from harbours known to have either of these species should be regularly hauled from the water and cleaned of biofouling. Solid and liquid waste from the cleaning process must be disposed on land, so as not to reintroduce fragments of the ascidians back into the ocean. This is because new colonies can regenerate from fragments. Care should be taken to make sure that

aquaculture product and equipment that has been exposed to a contaminated harbour is also clean before being moved to an uncontaminated harbour or aquaculture farm. If the spread of either of these species cannot be prevented, the primary objective is the early detection of their presence in a new location. This is because the probability of successful eradication depends upon the size of the invasive population. Small populations detected early have a higher likelihood of being eradicated. We recommend the deployment of small, polyvinyl chloride (PVC) settlement plates in high risk harbours for surveillance and early detection of *Botryllus schlosseri* and *Botrylloides violaceus*. We have also developed species-specific, TaqMan® quantitative, polymerase chain reaction (QPCR) assays which we recommend for highly sensitive, early detection and reporting of small and difficult-to-identify larvae and colony fragments. We also recommend a vigorous, targeted education campaign to marshal the power of the general public in the early detection of non-indigenous ascidians.

If either of these ascidians is detected in a new location, we recommend several options which may eradicate small populations. If the new colonies are detected on floating docks, we recommend that the docks are removed to land during the winter. If the new colonies are detected on wharf pilings, we recommend wrapping the pilings in plastic sheeting. This should be done after spring asexual reproduction has begun so that the presence of living colonies is easily detected, but before summer sexual reproduction has commenced. We have discovered that the timing of asexual growth and sexual reproduction can be predicted from water temperature. Sexual reproduction begins when the water temperature reaches ca. 11°C. Thus, encapsulation should be done before the water temperature reaches this threshold. Along the south coast of Newfoundland this means that encapsulation should generally be done sometime during the months of April or May. Vessels that are contaminated should be removed from the water and scraped. If this is not feasible, then the hulls can also be encapsulated in plastic as per the wharf pilings described above. Successful eradication and control require clear guiding principles and guidelines, political and financial commitment over the medium to long term, and high quality, local scientific information.

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This report was structured following the format of Kleeman (2009), which we gratefully acknowledge. The authors thank Jerry Ennis, Matt Pittman, Renee Boland, Will Coffey, Andrew Perry, Dave Poitras, and Mark O'Flaherty for conducting the SCUBA visual surveys and for the collection of specimens in Arnold's Cove and Belleoram harbours. We thank Central Diving Ltd. for providing commercial diving services in Belleoram harbour. We could not have completed our research without the help of Ashley Callahan, Laura Chomyshyn, Meredith Schofield, and Ainsley Latour. Gretchen Lambert kindly volunteered her time and taxonomic expertise to confirm our species identifications. Delores Wheeler and Connie Short assisted with purchasing and financial management, and Ken Langdon took care of our computing requirements. The cytochrome *c* oxidase I sequence reactions were carried out by the Genomics and Proteomics facility, CREAIT Network, Memorial University of Newfoundland.

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1.0 INTRODUCTION

The rapid spread of non-indigenous species (NIS) is a problem of global scale (Ruiz et al. 2000, Bax et al. 2001, Stachowicz et al. 2002a, Campbell et al. 2007). It has been suggested that anthropogenically driven changes in species distributions over the last 200-500 years have been as great as those which occurred naturally during the last ice age (di Castri 1989, in Williams and Grasholz 2008). In general, the problem is that some NIS can reach nuisance abundances (i.e., invasive species), threatening the sustainability of native ecosystems through the loss of key species or the reduction of species diversity (Trimble et al. 2009), and human economies and social systems (Blum et al. 2007, Dijkstra et al. 2007, Lutz-Collins et al. 2009, Fitridge et al. 2012).

Ecosystems and businesses on both the Atlantic and Pacific coasts of Canada have been threatened by aquatic invasive species (AIS) over the past couple of decades (Locke and Carman 2009, Switzer et al. 2011). Regionally, the bivalve aquaculture industry in New Brunswick, Prince Edward Island (PEI), and Nova Scotia has been affected severely over the last 10-15 years, resulting in shrinking profits and the closure of some farms (McKindsey et al. 2007, Locke and Carman 2009, Paetzold and Davidson 2010). The problem is serious. For example, the following statement was posted on the web site of the Aquaculture Association of Nova Scotia several years ago (http:// www.aansonline.ca/).

'Tunicates are now appearing at other previously uninfected sites in Nova Scotia and Prince Edward Island and could prove to be a major constraint to the survival and expansion of the shellfish industry. Efforts must be made to control the population of this invader on affected farms and contain its spread to other farms throughout the region.'

Although non-indigenous ascidians (NIA) (Tunicata, Ascidiacea) have been documented in Newfoundland since 1945 (U.S. Navy 1951), they have not yet reached invasive abundances. This provides the opportunity and obligation to do the necessary research to help prevent Newfoundland ecosystems and livelihoods from becoming severely affected by NIA.

Biofouling of bivalve aquaculture farms by NIA has been a global problem for at least 50 years (Yamaguchi 1975, Lützen 1999). Ascidians are common epibenthic animals occurring naturally in sub-tidal marine waters, particularly on hard substrates such as rocks, cliffs and the shells of other animals (Petersen and Riisgård 1992). They collect food particles using a mucous filter which traps particles as small as 1-2 µm in size with high efficiency (Jørgensen et al. 1984). Thus, they directly compete with cultured bivalves for food (Daigle and Herbinger 2009). Because they are essentially hollow animals with high water content, they can have high growth rates, allowing them to compete with bivalves for space and food (Svane and Young 1989, Lambert and Lambert 1998). Competition for space, as well as food, is the primary process by which NIA threaten the economic sustainability of bivalve farms (Stachowicz et al. 2002a)

In 2006, our research team, made up of scientists from the Department of Fisheries and Oceans Canada (DFO), Memorial University of Newfoundland (MUN), and the

Department of Fisheries and Aquaculture Newfoundland and Labrador (DFA), began a province-wide survey of harbours predicted to be at high risk for the establishment of NIA (Baines 2007). Once NIA were found, a research program began in 2008 on the genetics and population biology of *Botryllus schlosseri* and *Botrylloides violaceus*. This research included eradication experiments in the field, based upon our new, site-specific, scientific information. This information aided decisions as to when and where to target eradication trials, the choice of eradication technique, and guided decisions on the regional transfer of mussels from farms to processing plants.

Furthermore, because of the general lack of early warning techniques for marine invasive species, eradication or control measures are not generally applied until well after an invasion has occurred, reducing chances for success. Thus, we have also developed and tested species-specific, TaqMan®, quantitative, polymerase chain reaction (QPCR) assays for *Botrylloides violaceus,* to permit early detection of the presence of difficult-to-identify larval stages and colony fragments (Applin et al. in prep.). Similar assays are currently being developed by Applin for the early detection of *Botryllus schlosseri*.

This report contains information on the biology and ecology of the non-indigenous ascidians *Botryllus schlosseri* and *Botrylloides violaceus,* a summary of our research results, and recommendations arising from our research concerning the control of these fouling organisms in Newfoundland. Our team has gained experience in the identification of NIA in the field (Rapid Assessment and SCUBA surveys), designed gene probes for their identification, conducted eradication trials, and collected fundamental research information on the phenology of their growth, reproduction and recruitment. Thus, we offer practical experience and site-specific ecological data to the decision making process. We hope this report is useful to decision makers and managers, and serves as a guide for future research on NIA in Newfoundland.

2.0 DESCRIPTION OF *BOTRYLLUS SCHLOSSERI* AND *BOTRYLLOIDES VIOLACEUS*

2.1 TAXONOMY AND IDENTIFICATION

2.1.1 General biology

Ascidians (phylum Chordata, subphylum Tunicata) are benthic invertebrates that occur in solitary or colonial form depending upon species. They are primitive chordates, the closest living invertebrate relatives of vertebrates. Ascidians are filter feeders, deriving nutrients from phytoplankton, bacteria and other suspended organic particles (Millar 1971). Because the fleshy tunic is composed mostly of water, colonial ascidians grow rapidly when conditions are favourable (see Sections 2.3.3 and 2.3.4). Thus, they may overgrow surrounding plants and animals. Overgrowth also may increase hydrodynamic drag, so that fouled organisms may be dislodged by water currents (Clarke-Murray et al. 2012). In combination, these factors make ascidians aggressive competitors with other benthic plants and animals for space and food (Boyd et al. 1990, Nandakumar 1996). Thus, they are potentially disruptive to shellfish farms and to benthic communities

(Lambert 2002, Carver et al. 2006, Davis and Davis 2009, 2010, Locke and Carman 2009). In many parts of the world, including the Atlantic and Pacific coasts of Canada, invasive ascidians have made large operational and economic impacts on shellfish aquaculture operations (Switzer et al. 2011).

2.1.2 Origin and morphology

The Golden Star Tunicate (*Botryllus schlosseri)* is now found along the coasts of all continents except Antarctica (Fig. 1). It was discovered in eastern North America in about 1830 (Couthouy 1838), and was first identified in Newfoundland waters in 1945 (U.S. Navy 1951). The Violet Tunicate (*Botrylloides violaceus)* is native to the northwestern Pacific Ocean, most likely Japan (Oka 1927). Its global distribution is more limited than *B. schlosseri* (Fig. 2). *B. violaceus* was discovered in eastern North America in about 1945 (van Name 1945), and first identified in Belleoram, Newfoundland, in 2007 (Callahan et al. 2010). Although the area occupied by *B. violaceus* in Belleoram has increased from 2007 to 2012, it has not been observed elsewhere in Newfoundland as of April 2013.

Figure 1. Global occurrence of *Botryllus schlosseri* as reflected by information from several databases. A large portion of the data points for Canada came from the National Aquatic Invasive Species Monitoring Database (http://www.meds-sdmm.dfo-mpo.gc.ca/ais-eae, DFO 2013). The remaining points came from the Ocean Biogeographic Information System (http://iobis.org/mapper/, OBIS 2013). The map was downloaded from OBIS on 2 April 2013. As these maps depend upon voluntary uploading of primary research data, they should not be considered as being definitive descriptors of the complete global range. (Map credit: T. Wells)

Figure 2. Global occurrence of *Botrylloides violaceus* as reflected by information from several databases. (For data sources and caveats, see legend for Fig. 1) (Map credit: T. Wells)

Botryllus schlosseri colonies can be distinguished from other colonial ascidians by the conspicuous, star-shaped arrangement of individual zooids within a translucent, firm, thin, fleshy matrix or tunic (Berrill 1950) (Fig. 3). Found in an array of colours and pigment patterns (Ma 2012), colonies are typically densely packed into a mat that covers the underlying substrate.

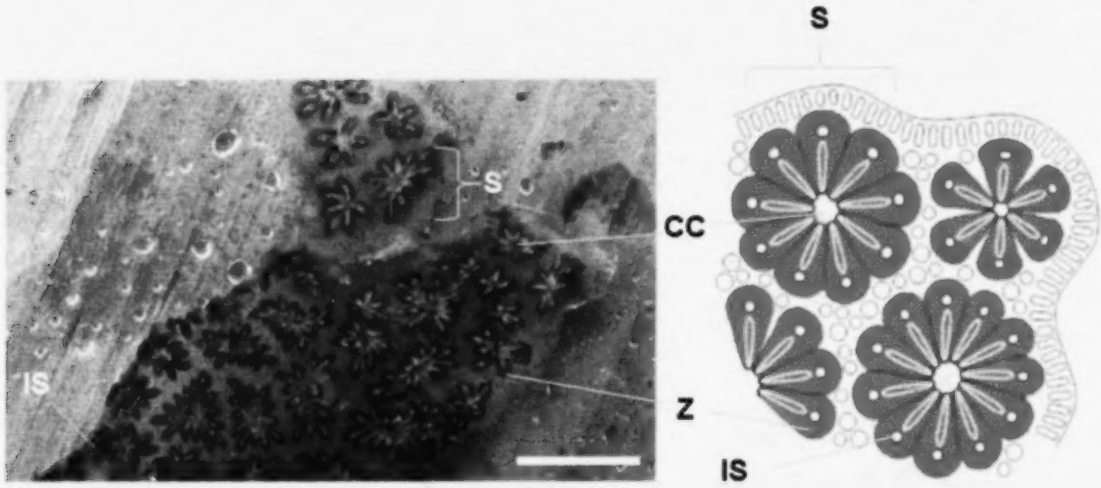

Figure 3. A colony of *Botryllus schlosseri*. Note the stellate arrangement of individual zooids into separate systems making up the colony. Z = zooid, IS = inhalent siphon of a zooid, CC = the common cloaca of a system which serves as the exhalent siphon, and S = a system of zooids. Scale bar in photograph = ca. 1 cm. The drawing is not to the same scale as the photograph. (Photograph and drawing credit: K.C.K. Ma)

B. schlosseri reproduces asexually by fragmentation and budding (see Section 2.5.1) (Lauzon et al. 1993), and sexually by the fertilization of brooded eggs that hatch into free-swimming larvae (see Section 2.5.2) (Milkman 1967, Satoh 1994). Newfoundland has been declared to be at high risk for invasion by *B. schlosseri* (Therriault and Herborg 2008).

Botrylloides violaceus can be distinguished from other colonial ascidians by the distinct ridge or track-like patterns of zooids on the surface of the fleshy tunic in which they are embedded (Carver et al. 2006) (Fig. 4). *B. violaceus* has fewer colour morphotypes than does *Botryllus schlosseri*, and is typically uniformly coloured in shades of orange, burgundy, dull pink, lavender or purple (Lambert and Lambert 2003). Colonies are often densely packed, with mats or lobes readily overgrowing the underlying substrate (see Section 2.3.3). *B. violaceus* is on the Canadian list of highly invasive species, and Newfoundland has been declared at high risk for invasion (Therriault and Herborg 2008). In general, *B. violaceus* seems to grow more rapidly than *B. schlosseri*, particularly at temperatures < 10°C (Epelbaum et al. 2009a). Also, *B. violaceus* is the more aggressive competitor for space, primarily due to seasonally early recruitment and lower susceptibility to predators (Osman and Whitlach 1995).

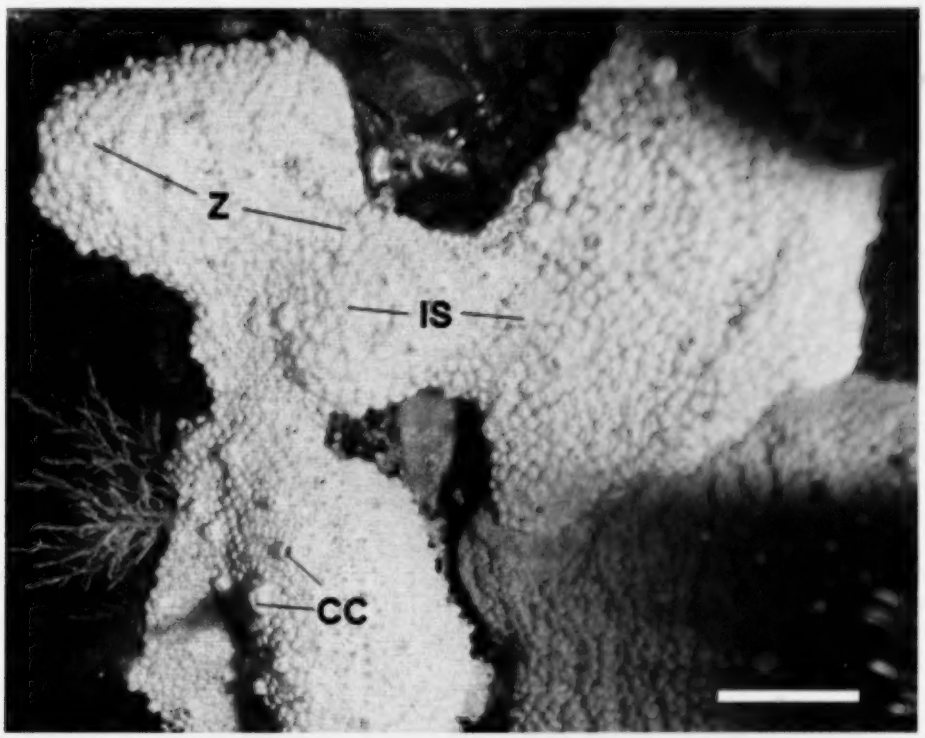

Figure 4. Two colonies of *Botrylloides violaceus* of differing colour morphotype. Z = zooid, IS = inhalent siphon of a zooid, CC = the common cloaca which serves as an exhalent siphon. Scale bar = ca. 1 cm. (Photograph credit: R. O'Donnell)

2.1.3 Genetics

A great deal is known of the genetics of *Botryllus schlosseri*, while less is known about *Botrylloides violaceus*. *B. schlosseri* has high genetic variability on all spatial scales, from tens of meters to global (Grosberg 1991, Yund and Stires 2002, López-Legentil et al. 2006, Callahan et al. 2010, Bock et al. 2012). Although little research has been done on the genetic basis of the many colour morphotypes, it is thought that the colour morphs are genetically based and inherited in a Mendelian fashion (Milkman 1967, Sabbadin 1971). However, the high genetic variability of *B. schlosseri* suggests that there may be cryptic species present (Bock et al. 2012). Work we have done suggests high genetic variability of the cytochrome *c* oxidase I gene (COI) of *B. schlosseri* in Newfoundland coastal waters, and that local populations have likely originated from specific harbours on the Mediterranean Sea and Atlantic Ocean coasts of France and Spain (Callahan et al. 2010).

Botrylloides violaceus has much lower genetic variability than *Botryllus schlosseri* (Callahan et al. 2010, Bock et al. 2011). So far, only two haplotypes of the COI gene have been documented from many sites along the east coast of North America (Callahan et al. 2010, Bock et al. 2011). This is in contrast to *B. schlosseri*, which can have 4 haplotypes within a single harbour (López-Legentil et al. 2006). This also suggests that *B. violaceus* may have been introduced to North America much more recently than *B. schlosseri* (Dijkstra et al. 2007).

2.2 DISTRIBUTION

2.2.1 Global

Botryllus schlosseri is found from sub-polar waters to the tropics around the world (Ben-Shlomo et al. 2006) (Fig. 1), and has wide temperature and salinity tolerances (10-25°C and 14-38) (Dijkstra et al. 2008; Epelbaum et al. 2009a). It is probably of Mediterranean Sea origin, and has been transported around the world on ship hulls (Ben-Shlomo et al. 2006).

Botrylloides violaceus has been reported on both coasts of North America (Carver et al. 2006), as well as from Italy, the United Kingdom, Ireland, the Netherlands, and Australia (Zaniolo et al. 1998, Gittenberger 2007, Minchin 2007, Perez-Portela et al. 2009) (Fig. 2). While *B. violaceus* has been reported from Mexico to Alaska on the west coast (Lambert and Lambert 2003), on the east coast it has been detected primarily from Connecticut to Newfoundland (Carver et al. 2006, Callahan et al. 2010). Young colonies of *B. violaceus* have wide temperature and salinity tolerances (5-25°C and 20-38) (Epelbaum et al. 2009a).

2.2.2 Regional

In the northwest Atlantic Ocean, *Botryllus schlosseri* is present from Cape Hatteras, North Carolina to the island of Newfoundland (Fig. 1) (reviewed in Ma 2012). *B. schlosseri* is common in eastern Canada, including the islands of St. Pierre and Miquelon (France), the Magdalen Islands (Québec), New Brunswick, PEI, and Nova Scotia (reviewed in Ma 2012). Although reported in the Gulf of St. Lawrence a hundred

years ago, *B. schlosseri* was rarely reported in the Maritime provinces thereafter, having been first reported in PEI in 2001 (Locke et al. 2007). *B. schlosseri* has been an invasive pest to bivalve aquaculture farms in PEI and Nova Scotia, but recently has been replaced by invasive solitary ascidian species (Locke and Carman 2009).

Botrylloides violaceus probably became established in the Gulf of Maine in the early 1970s via oyster culture (Dijkstra et al. 2007). It subsequently became a major competitor for space in the subtidal zone of the Gulf of Maine (Dijkstra et al. 2007). *B. violaceus* monocultures dominate the subtidal zone of Buzzards Bay, Massachusetts, and many other bays in New England, replacing *Mytilus edulis* and other NIA (Rajbanshi and Pederson 2007). In eastern Canada, *B. violaceus* was first reported in PEI in 2002 (Locke et al. 2007) and in Newfoundland by our group in October, 2007 (Callahan et al. 2010). It now occurs in many locations on the coasts of both PEI and Nova Scotia (Bock et al. 2011, Sephton et al. 2011). Like *Botryllus schlosseri, B. violaceus* is currently present in the northwestern Atlantic Ocean from Cape Hatteras, North Carolina, to the island of Newfoundland (Fig. 2).

2.2.3 Local

Botryllus schlosseri was first reported in Argentia, Newfoundland, in 1945 (U.S. Navy 1951). It was not reported again until 1975, in Bonne Bay and St. Paul's Inlet, on the west coast of the island (Hooper 1975). About 30 years later, we found *B. schlosseri* in several harbours along the south coast of Newfoundland (Callahan 2010). As of December 2012, *B. schlosseri* was present in 23 harbours in Newfoundland, primarily along the south coast (Ma 2012, Fig. 5).

Botrylloides violaceus has been found in only a single harbour on the south coast of Newfoundland (Fig. 5). This suggests it has only recently been introduced. However, its population has expanded substantially within Belleoram harbour since 2007, suggesting that it may spread to other harbours in the future.

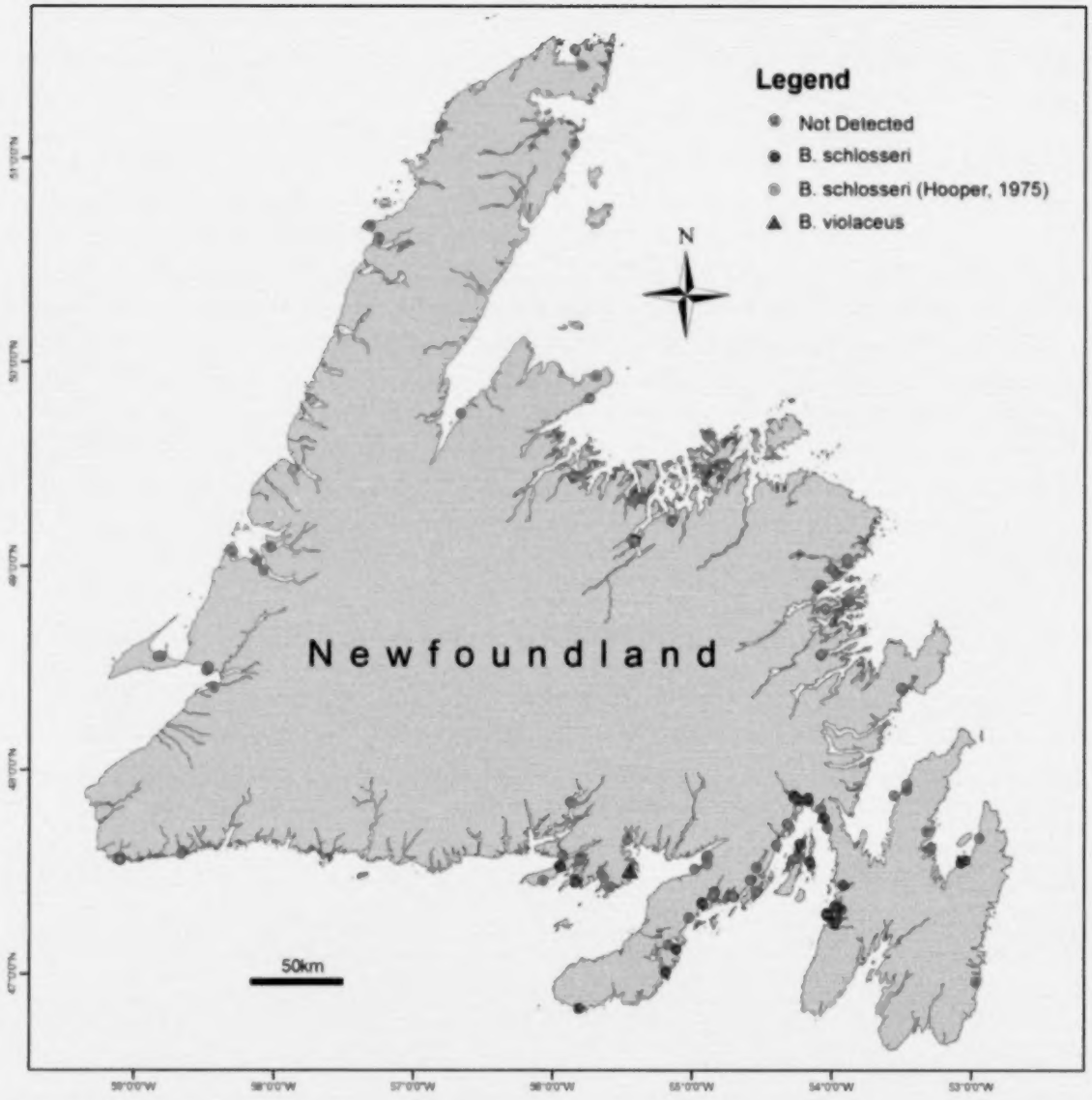

Figure 5. Map of the island of Newfoundland showing those locations at which *Botryllus schlosseri* and *Botrylloides violaceus* have been found as of April 2013. Green circles indicate those locations which have been surveyed (2006-2012) without detecting either species. The two orange circles are populations first reported by Hooper (1975) that no longer exist (B. Hooper, pers. comm.). (Map credit: T. Wells).

2.3 HABITAT AND GROWTH

2.3.1 Depth range

Botryllus schlosseri has been found from the intertidal zone to 75 m or more (Rinkevich et al. 1998), while *Botrylloides violaceus* occurs primarily at depths < 50 m (Carver et al. 2006). However, most colonies are in the subtidal zone. In Arnold's Cove, our SCUBA surveys revealed intertidal and subtidal colonies of *B. schlosseri,* but none on the sea floor.

2.3.2 Preferred substrates

Preferred substrates of both species include naturally occurring pebbles, rocks, and boulders, eelgrass, kelp, bivalve and crustacean shells, as well as many man-made materials, including wharf pilings and sub-wharf ballast rocks, the submerged surfaces of decks and barrels of floating docks, buoys, vessel hulls, pipes, cables, chains, etc. We found that larvae of *Botryllus schlosseri* recruit to settlement plates made of polyvinyl chloride (PVC) at higher rates than to plates made of untreated softwood or aluminum-encased softwood (Ma 2012).

2.3.3 Asexual growth

Botryllus schlosseri and *Botrylloides violaceus* colonies grow asexually to approximately 10 or 15 cm in diameter, respectively. When space is limited, both species may develop lobes extending into the water column. The lobes of *B. violaceus* may exceed 5 cm in length.

Botryllus schlosseri colonies can reach a maximum size of ca. 1500 zooids, but typically range from 100-1000 zooids (Grosberg 1988). *Botrylloides violaceus* generally grows to a larger size (Carver et al. 2006). Positive asexual colony growth in both species occurs where bud production exceeds the number of adult zooids resorbed during each blastogenic cycle (Berrill 1950, Mukai et al. 1987). Prior to sexual maturation, asexual growth rates in both species are maximal when the length of each blastogenic cycle is at its shortest (e.g., one complete cycle in 5-7 d) (Grosberg 1988, Westerman 2007). With a maximum budding rate of 3-4 buds per zooid in each blastogenic cycle, *B. schlosseri* colonies can attain a size of > 1000 zooids in 6-7 cycles, or ca. 30-50 d at 18°C (Grosberg 1988).

Asexual growth rates (i.e., the number of zooids produced time^{-1}) in botryllid colonies are exponential until the onset of sexual maturation (Yamaguchi 1975, Grosberg 1988, Epelbaum et al. 2009a). Once a colony reaches sexual maturity, asexual growth proceeds at a much slower rate, because energy is diverted from the production of additional buds to the production of energetically expensive eggs. Consequently, asexual colony growth rates and terminal colony size are strongly dependent on the seasonal timing of sexual reproduction. For example, at a given food level, semelparous colonies (i.e. those reproducing only once before dying) that reproduce earlier in life are typically much smaller than iteroparous colonies (i.e. those reproducing more than once before dying) that reproduce later in life (Grosberg 1988). As in other benthic organisms, density-dependent competition for space and food may also limit colony growth. The

blastogenic cycling rate is a useful index of colony-wide, asexual growth rates, since asexual growth rates are coupled to the length of the blastogenic cycle (Lowen et al. in prep.) (Fig 6).

2.3.4 Seasonal pattern of growth

The seasonal timing of colony growth is strongly dependent on the prevailing temperature regime (see Section 2.4.1). Thus, the growing season of many ascidian species is progressively shorter with colder sea water temperatures (Millar 1952). In the Mediterranean Sea, *Botryllus schlosseri* colony growth proceeds almost year round (Rinkevich et al. 1998). In southerly temperate environments, the annual window for colony growth is ca. 10 months (e.g. Chadwick-Furman and Weissman 1995, Monterey Bay, California). In contrast, the annual window for colony growth in more northerly temperate environments is ca. 8 months (e.g. Grosberg 1988, Buzzards Bay, Massachusetts). Near the northern limit of its present distributional range in the northwestern Atlantic Ocean, in Arnold's Cove, Newfoundland, the length of the growing season is reduced to only 5 months (Lowen et al. in prep.) (Fig. 6) (see Section 2.4.1).

As the growing season becomes increasingly time constrained with increasing latitude and lower water temperatures, smaller semelparous colonies of *Botryllus schlosseri* are more frequent than larger iteroparous colonies (Lowen et al. 2011, 2012). In temperate waters, the time at which larvae settle in relation to the beginning or end of the growing season also affects asexual growth rates of the subsequent colonies. Colonies originating at the beginning of the growing season typically grow more slowly in the colder spring and early summer than those originating during higher water temperatures in mid-summer (Grosberg 1988, Chadwick-Furman and Weissman 1995, Lowen et al. 2011, 2012). Moreover, colonies originating at the end of the growing season may enter an overwintering, dormant phase, where asexual growth effectively ceases (Grosberg 1988). During this time, colonies may undergo reversible regression, or irreversible degeneration and death (Brunetti et al. 1980). For colonies that survive the winter, colony regeneration and asexual growth resumes with the onset of the next growing season.

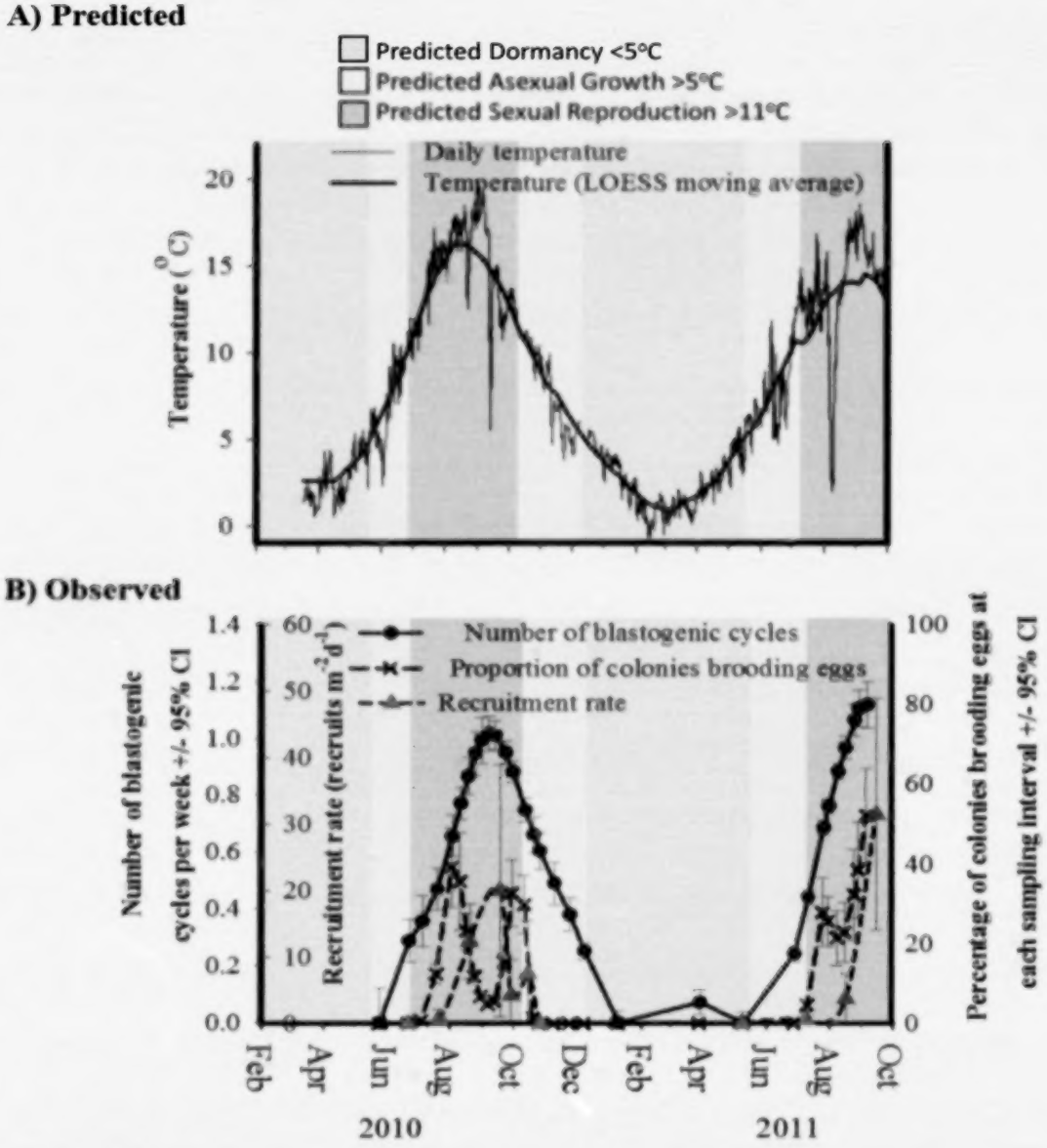

Figure 6. The annual cycle of asexual growth, sexual reproduction, and larval recruitment of *Botryllus schlosseri* in Arnold's Cove, Newfoundland. (A) 20-month time series of daily water temperatures in Arnold's Cove harbour. (B) Matching 20-month time series of asexual growth rate (number of blastogenic cycles wk^{-1}), sexual reproduction (% of colonies brooding eggs), and larval recruitment rate (recruits m^{-2} d^{-1}). Background shading indicates predicted temporal windows for overwintering dormancy (blue), asexual growth (tan), and sexual reproduction (grey). The temporal windows are based upon literature information on these life history stages for temperate-zone populations (see text for references). (Figure credit: J.B. Lowen)

2.4 FACTORS AFFECTING GROWTH AND REPRODUCTION

2.4.1 Temperature control of the life cycle

Asexual growth of *Botryllus schlosseri* and *Botrylloides violaceus* is suspended below temperatures of 5-6°C (Brunetti et al. 1980, Grosberg 1988, Westerman 2007), while sexual reproduction takes place at temperatures > 11-13°C (Brunetti 1974, Brunetti et al. 1980, 1984, Grosberg 1988, Epelbaum et al. 2009a, Westerman 2007, Westerman et al. 2009). Based on these limiting temperatures, Lowen et al. (2011, 2012) predicted temporal windows for asexual growth, sexual reproduction, and overwintering dormancy in *B. schlosseri* in Arnold's Cove, Newfoundland, from local temperature records (Fig. 6). By comparing the predicted windows with those observed, Lowen et al. (2011, 2012) determined; i) the degree to which the growing season was time constrained by temperature and ii) whether *B. schlosseri* had undergone any physiological adaptation to allow asexual growth and sexual reproduction to proceed below the published temperature thresholds for these processes.

Since *Botryllus schlosseri* releases larvae at the end of the blastogenic cycle (see Section 2.5.2), the window for larval settlement can also be predicted from the temperature threshold for sexual reproduction (Ma 2012). In *Botrylloides violaceus*, hatched larvae are retained within the colony and are not released at the termination of each blastogenic cycle (Westerman 2007). In addition, spawning is not as tightly synchronized within a colony as in *B. schlosseri* (Mukai et al. 1987). Both of these factors make it more difficult to predict the onset of larval settlement in *B. violaceus* than in *B. schlosseri* (Westerman 2007, Westerman et al. 2009).

For the Arnold's Cove population, the predicted windows for asexual growth, sexual reproduction, and larval settlement matched closely with those observed (Lowen et al. 2011, 2012) (Fig. 6). Thus, there has been no physiological adaptation to allow asexual growth, sexual reproduction, or larval settlement to commence below the published limiting temperatures for these key life-cycle events (Lowen et al. 2011, 2012, Ma 2012). Consequently, the life cycle of *Botryllus schlosseri* in sub-arctic Newfoundland waters is more severely time constrained by temperature than in temperate waters (Lowen et al. 2011, 2012, Ma 2012). Thermal time constraints ultimately limit population growth in ectotherms, and may explain why *B. schlosseri* has not yet become an invasive pest in Newfoundland. However, heating of the ocean due to global climate change may result in a longer growing season and thus greater annual population growth of *B. schlosseri* and *B. violaceus* (Stachowicz et al. 2002b). Interestingly, *B.schlosseri* exhibits earlier sexual maturation (i.e., after fewer blastogenic cycles) in sub-arctic waters than in temperate waters, thereby shortening its generation time (Lowen et al. in prep). This strategy is adaptive, as fitness in ectotherms is ultimately enhanced by producing as many generations as possible as the growing season becomes increasingly time constrained by temperature (Kivela et al. 2009).

Rates of asexual reproduction and egg brooding also increase with increasing temperature in both *Botryllus schlosseri* (Sabbadin 1958, in Grosberg 1991, Epelbaum et al. 2009a) and *Botrylloides violaceus* (Westerman 2007). Corroborating these

findings, Lowen et al. (2011, 2012) observed a significant positive correlation between temperature and rates of asexual reproduction and the proportion of colonies brooding eggs (Fig. 6). Larval settlement rates also increased with increasing temperature, peaking in mid-September when summer seawater temperatures were maximal (Ma 2012). Furthermore, the length of the blastogenic cycle for a given temperature was in close agreement with that observed in laboratory and field studies of colonies in temperate waters. The length of the blastogenic cycle in Newfoundland increased from 0.5 cycles wk^{-1} at 10°C to one cycle wk^{-1} at ca. 17°C (Lowen et al. 2011, 2012), as observed elsewhere (Chadwick-Furman and Weissman 1995, Grosberg 1988, Westerman 2007).

Overall, our observations emphasize the role of temperature in regulating the life-cycle of *Botryllus schlosseri*. By predicting the seasonal timing and rates of key life-cycle events from local temperature data, one can determine where and when to target eradication efforts in any location in Newfoundland (see Section 4.1.3).

Overwintering survival of colonies originating in the fall was surprisingly high in the sub-arctic waters of Newfoundland (i.e., ca. 90%) (Lowen et al. 2011, 2012). That *B. schlosseri* colonies survived the winter is also reflected in their year-round abundance (Ma 2012). Survival to sexual maturity the following summer was also similar to that observed in temperate populations (Grosberg 1988, Chadwick-Furman and Weissman 1995). Thus, reproductively viable populations of *Botryllus schlosseri* can survive from year-to-year in sub-arctic waters.

2.4.2 Salinity

As befits their invasive abilities, the environmental niches of *Botryllus schlosseri* and *Botrylloides violaceus* are wide. *B. schlosseri* can tolerate salinities from at least 14-38 (Brunetti et al. 1980, Epelbaum et al. 2009a), while *B. violaceus* has slightly narrower salinity limits (i.e., 20-38) (Dijkstra et al. 2008; Epelbaum et al. 2009a). Maximum growth rates of *B. schlosseri* occur at salinities > 20, while maximum growth rates of *B. violaceus* occur at salinities > 25 (Epelbaum et al. 2009a). Both species experience 100% mortality at a salinity of 5 (Dijkstra et al. 2007). In general, adults have wider salinity tolerance limits than larvae (see review by Carver et al. 2006).

2.4.3 Predators

Few naturally occurring predators of *Botryllus schlosseri* and *Botrylloides violaceus* are known. Recruits of *B. schlosseri* have been observed to be eaten by fish, such as cunner (*Tautogolabrus adspersus*) and winter flounder (*Pseudopleuronectes americanus*), several species of small snails (*Mitrella lunata, Anachis lafresnayi*) (Osman and Whitlach 1995, 2004), sea urchins (*Strongylocentrotus droebachiensis, Strongylocentrotus franciscanus*), and several species of sea stars (*Dermasterias imbricata, Hermissenda crassicornis, Patiriella brevispina, Petricia vernicina, Tosia australis, Coscinasterias calamaria*) (Keough and Butler 1979, Epelbaum et al. 2009b). After colonies reach ca. 3 weeks of age, predation mortality becomes lower. Recruits of *B. violaceus* may also be vulnerable to browsing fish (Yamaguchi 1975) and are eaten by chitons (*Mopalia mudosa*) (Nydam and Stachowicz 2007), sea urchins, and sea stars

(Keough and Butler 1979, Epelbaum et al. 2009b). However, predatory mortality of *B. violaceus* is low after only 1 week of settlement (Osman and Whitlach 1995, 2004). The few known predators of adult *B. schlosseri* and *B. violaceus* include snails, flatworms (*Cycloporus papillosus, C. japonicus*), spider crabs (*Libinia emarginata*), nudibranchs (*Goniodoris castenata*), sea urchins, sea stars, and winter flounder (review by Carver et al. 2006, Dijkstra et al. 2007, Epelbaum et al. 2009b). However, in the Gulf of Maine, green sea urchins have been found to prey upon native ascidians but not on *B. violaceus* (Simoncini and Miller 2007). We have also found sea urchins to be ineffective predators of *B. violaceus* in Newfoundland (McKenzie et al., unpubl.). This discrepancy between predation rates determined in the laboratory vs. the field may be due to the availability of preferred, non-ascidian prey in the field (Epelbaum et al. 2009b). The preference for other prey may account for why sea urchins and sea stars are not thought to significantly regulate botryllid recruitment in nature (Keough and Butler 1979, Epelbaum et al. 2009b). This factor should be taken into account when considering the use of introduced predators as potential control agents of NIA in the field.

2.4.4 Light

The larvae of most ascidian species are photonegative at settlement, choosing dark-coloured surfaces in the laboratory and the shaded, underside of surfaces in the field (Svane and Young 1989, Lützen 1999, Lambert and Lambert 2003, Feng et al. 2010). This effect may be subtle however, as Olson (1983) found that the larvae of colonial ascidians have a preferred illumination level of ca. 100 $\mu E\ m^{-2}\ s^{-1}$, which occurs at ca. 15 m depth in many coastal waters.

Light may have a direct effect on the survival of juvenile and adult ascidians. Bingham and Reitzel (2000) used various filters to manipulate the exposure of juvenile and adult *Corella inflata* to naturally occurring ultraviolet-A (UVA), ultraviolet-B (UVB) and visible light (photosynthetically active radiation, PAR) irradiation. They found that PAR alone had the greatest effect on survival, with an order of magnitude decrease in larval settlement rate after only 3 h of exposure to full spectrum sunlight. The mechanism of the effect of PAR on ascidian survival and larval settlement is unknown.

Little is known about the effect of light on *Botryllus schlosseri* and *Botrylloides violaceus*. *B. schlosseri* possesses limited UV shielding and prefers to settle in areas not exposed to direct sunlight. Grosberg (1988) observed colony mortality following exposure to light from a microscope for only 20 min. We have found that focused white light from a thermally isolated, fibre optic, microscope light source killed larvae of *B. schlosseri* within 1 min, while only 1 h of exposure to ambient sunlight, on a cloudless day, resulted in the mortality of adult colonies in the field (Lowen unpubl.).

2.5 REPRODUCTION AND DISPERSAL

2.5.1 Asexual reproduction

Botryllus schlosseri and *Botrylloides violaceus* colonies are hermaphroditic, composed of asexually produced zooids (Berrill 1950, Mukai et al. 1987). All zooids in a colony simultaneously undergo asexual replacement during the blastogenic cycle, by palleal

budding. Replacement is termed blastogenesis. The rate of blastogenesis is inversely related to water temperature (Sabbadin 1958, in Grosberg 1991, Epelbaum et al. 2009a). At the end of the blastogenic cycle, when the primary palleal buds replace the zooids to which they were attached, the new generation of zooids opens its siphons and begins to feed.

Asexual reproduction also takes place by fragmentation (Bullard et al. 2007). Fragmentation may occur as a result of several physical processes, including wave turbulence, tidal currents, propeller or wake turbulence, manual removal of colonies from surfaces, and high pressure water jets (Bullard et al. 2007, Paetzold and Davidson 2010, Arens et al. 2011, Switzer et al. 2011). Once fragments attach to a new surface, they may regenerate whole colonies. As long as fragments include ampullae, colonies may even regenerate from fragments of tunic by vascular budding (Rinkevich et al. 2007, Kürn et al. 2011). Vascular budding is also likely the process that takes place during the spring regeneration of overwintering, regressed colonies of *Botryllus schlosseri* (Burighel et al. 1976). Vascular budding is the only known case of whole-body regeneration among the chordates (Kürn et al. 2011). In fact, ascidians possess among the greatest post-embryonic regenerative plasticity of any metazoan (Kürn et al. 2011). Both *B. schlosseri* and *Botrylloides violaceus* have been shown to regenerate from fragments (Bullard et al. 2007). Fragmentation must be minimized if eradication measures are to be effective (Paetzold and Davidson 2010).

2.5.2 Sexual reproduction

In colonies of *Botryllus schlosseri* and *Botrylloides violaceus*, ovaries and testes (one pair zooid^{-1}) appear in the palleal buds while they are still attached to the parental zooids (Milkman 1967, Mukai et al. 1987, Manni et al. 1994, Satoh 1994). During takeover at the end of the blastogenic cycle, the new generation of zooids opens its siphons, and ovulation occurs (Grosberg 1988, Chadwick-Furman and Weissman 1995, Yund et al. 1997, Westerman 2007, Westerman et al. 2009). With the onset of the sexual blastogenic cycle, all of the zooids in the colony reach sexual maturity synchronously. The ova of *B. schlosseri* are released into the atrial chamber of the parents, while those of *B. violaceus* are moved into a brood pouch, just posterior of the egg follicles (Mukai 1977). In both species, eggs are fertilized within 1 d, presumably by sperm from nearby colonies brought in with the incurrent flow of water. Self-fertilization is probably rare as testes in the parental zooid ripen after the ova (Mukai et al. 1987, Manni et al. 1994, Stewart-Savage and Yund 1997). There may also be chemical or genetic barriers to self-fertilization (Scofield et al. 1982, Grosberg 1991).

In *Botryllus schlosseri*, fertilized ova are retained in the zooids within egg sacs. Embryogenesis is completed just prior to the regression of zooids at the end of the blastogenic cycle. Larvae are subsequently released from the exhalent siphons of the colony, after which they swim for 3-12 h before settling (Grosberg 1988). The larvae settle on hard substrates, often only a short distance from the parental colony. Within 1 d they resorb the tail, and metamorphose into the first zooid of a colony, known as the oozooid (Saito and Okuyama 2003). If colonies are iteroparous, sexual reproduction may continue in concert with the asexual blastogenic cycle for up to a further eight

cycles, thus producing up to eight clutches of eggs (Grosberg 1988, Chadwick-Furman and Weissman 1995).

The egg of *Botrylloides violaceus* is fertilized while in the brood pouch (Mukai et al. 1987). Developing embryos survive the disintegration of the parental zooids, with competent larvae leaving the colony a month or more later. Thus, it is common to find developing larvae of distinctly differing ages within a single colony of *B. violaceus*, each age group released by successive generations of parental zooids (Mukai et al. 1987). Larvae are not released synchronously from a colony as in *B. schlosseri*, but aperiodically. Because of the prolonged gestation period in the brood sack, the larvae are large when released, ca. 3.0 mm in total length (Mukai et al. 1987).

Clutch size in *Botryllus schlosseri* is dependent on the number of eggs per zooid (1-4) and the number of zooids in a mature colony (i.e., 50-1500 zooids colony^{-1}) (Grosberg 1988). Semelparous colonies exhibit higher reproductive effort, producing more eggs zooid^{-1} than iteroparous colonies (Grosberg 1988). Consequently, clutch size is greater in semelparous than in iteroparous colonies for a given colony size. However, the lifetime reproductive output of iteroparous colonies (up to a maximum of 8000 eggs colony^{-1}) is up to 3-4 times greater than semelparous colonies (Grosberg 1988). This is because iteroparous colonies defer reproduction, thereby reaching a larger colony size, after which they release eggs during up to 8 blastogenic cycles (Grosberg 1988).

2.5.3 Natural dispersal

Since botryllid larvae have a limited free-swimming period, they disperse only short distances. In relatively enclosed harbours, most larvae settle within 1 m of their parent colony (Grosberg 1987), and gene flow estimates show spatial autocorrelation over < 5 m (Yund and O'Neil 2000). In tidal estuaries, genetic studies indicate that larvae may be transported up to 1.5 km from their parent colony (Yund and Stires 2002). Thus, the transport of larvae is unlikely at scales > 1-2 km.

Two possibilities for non-larval dispersal are fragmentation and rafting. Natural colony fragmentation is rare, ca. 2% of the population (Grosberg 1988, Lowen unpubl.). However, colony fragments of *Didemnum vexillum* can survive for up to 40 days (Bullard et al. 2007), and thus can be dispersed by currents over much larger distances than short-lived larvae. Rafting is common in colonial ascidians and possibly a more significant mechanism of non-larval dispersal than fragmentation. In Newfoundland coastal waters, many colonies can be found attached to mussel shells and kelp fronds (Ma 2012) (Fig. 7). When the kelp fronds become detached from their stipe due to currents, ice scour, storms, or bryozoan fouling, they may drift great distances, providing a vector for long-distance transport of colonies. Eelgrass rafts may also be an important naturally occurring vector.

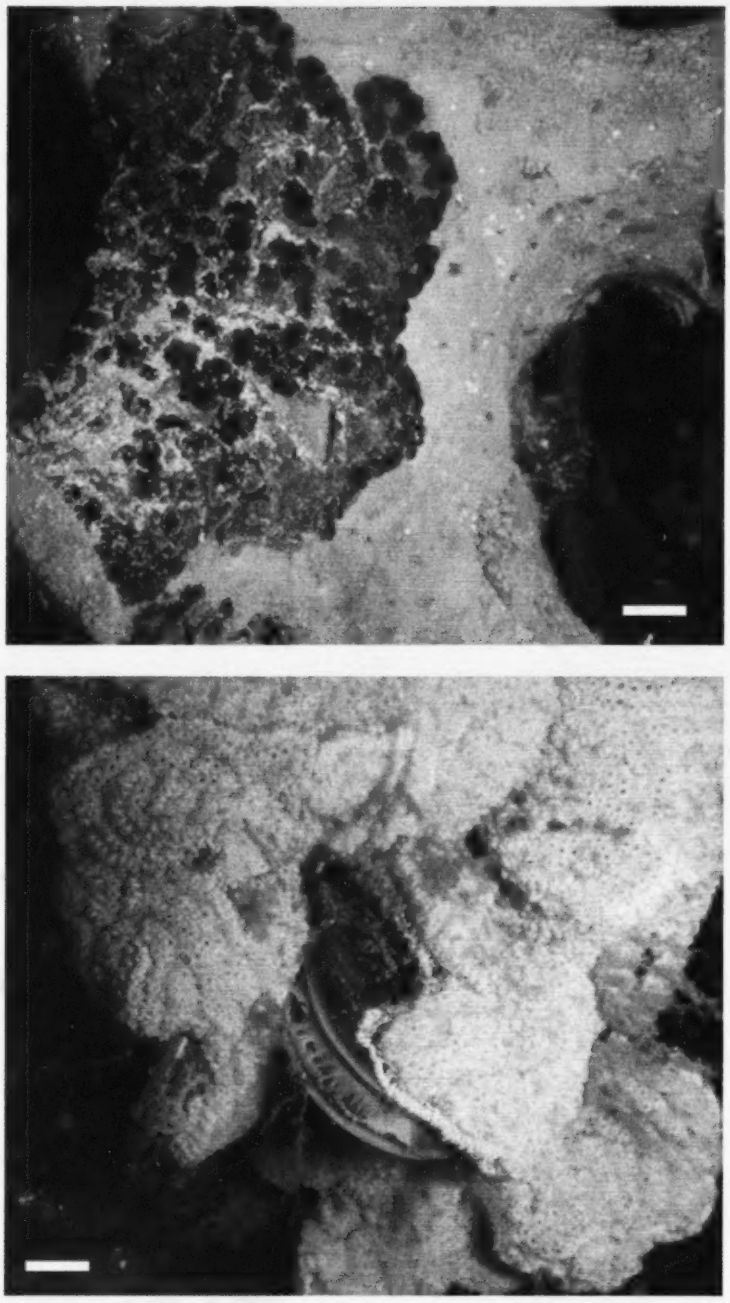

Figure 7. Common naturally occurring substrates for the attachment of *Botryllus schlosseri* and *Botrylloides violaceus*. (A): A colony of *B. schlosseri* attached to a frond of the kelp *Saccharina longicruris* in Arnold's Cove, Newfoundland. (Photograph credit: R. O'Donnell) (B): Colonies of *B. violaceus* attached to shells of the mussel *Mytilus edulis* in Belleoram, Newfoundland. Scale bars = ca. 1 cm. (Photograph credit: R. O'Donnell)

2.5.4 Anthropogenic dispersal

It is generally believed that colonial ascidians are transported long distances by their attachment to vessel hulls and in sea chests (Ramsay et al. 2008, Davis and Davis 2009, Coutts et al. 2010, Clarke-Murray et al. 2012). Since NIA are already present on the island of Newfoundland, regional spread by local vessels is likely a significant vector (Acosta and Forrest 2009). During our SCUBA surveys, we have frequently encountered inshore fishing vessels and pleasure craft heavily fouled by *Botryllus schlosseri* and *Botrylloides violaceus* (Fig. 8). If these vessels are moored in an ascidian-free harbour during the time of larval release (i.e., August-October), the likelihood of spreading to the new harbour is high. Ascidian species have differential abilities to cling to moving vessels, with the colonial botryllids among the most tenacious at vessel velocities > 10 nautical miles h^{-1} (Coutts et al. 2010, Clarke-Murray et al. 2012).

Fouled buoys and drifting wharf timbers can also transport colonies long distances. In addition, colonies of *Botryllus schlosseri* and *Botrylloides violaceus* have been found attached to lobsters, crabs, mussels, oysters, clams, and scallops (Bernier et al. 2009, Morris et al. 2009, Carman and Grunden 2010). Fouled crustaceans may be transported long distances, either by their own efforts or during commercial transport. In addition, the equipment used for transport, such as lobster cages, may be vectors for the regional movement of NIA. Fouled shellfish may serve as vectors during harvesting and transportation processes (Locke et al. 2007, Izquierdo-Muñoz et al. 2009, Carman et al. 2010, Paetzold and Davidson 2010).

There is active interprovincial traffic in the buying and selling of coastal fishing vessels. In particular, fish harvesters on the south coast of Newfoundland frequently buy used vessels from PEI and Nova Scotia, provinces known to have NIA (Fig. 9). These vessels are possible vectors for the original introduction of *Botryllus schlosseri* and *Botrylloides violaceus* into Newfoundland. There are at present no requirements for hull cleaning before the interprovincial movement of coastal vessels (Canada Shipping Act (2001), available at: http://laws-lois.justice.gc.ca/eng/ acts/C-10.15/FullText.html). Aquaculture support vessels do have requirements for hull cleaning before movement between provinces (provincial Health and Safety regulations), and require certification from the provincial veterinarian before sailing. In anticipation of the International Maritime Organization's *Biofouling Guidelines* being mandatory, vessels are encouraged to implement voluntary measures for the management of biofouling to minimize the transfer of invasive species.

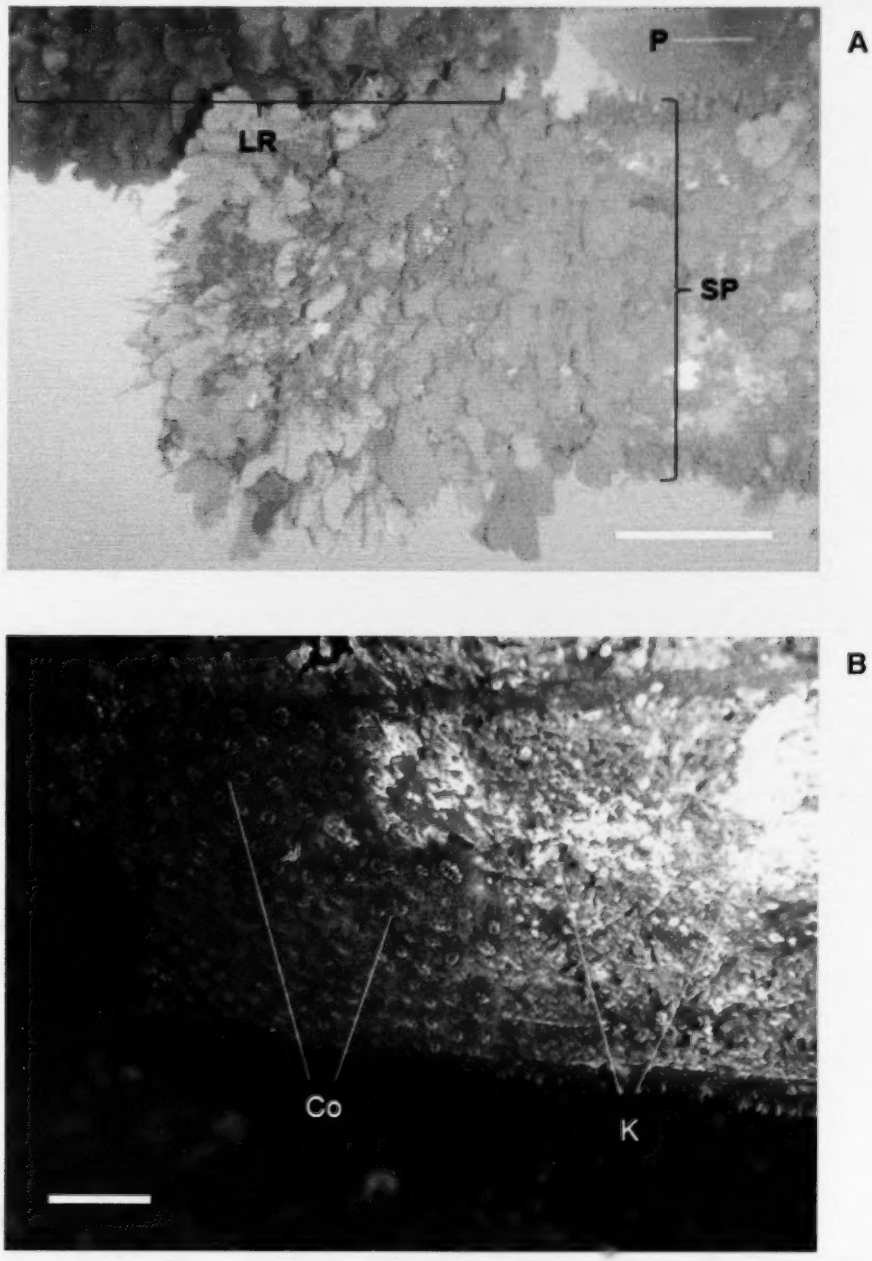

Figure 8. Hull fouling by *Botryllus schlosseri* and *Botrylloides violaceus*. (A): Fouling of the rudder and stern post of a coastal vessel in Belleoram, Newfoundland, by colonies of *B. violaceus* of two colour morphotypes. LR = lower rudder, SP = stern post, P = propeller. Scale bar = ca. 10 cm. (Photograph credit: R. O'Donnell) (B): Fouling of the keel of a recreational sail boat in Foxtrap Marina, Newfoundland, by colonies of *B. schlosseri*. K = keel, Co = colony. Scale bar = ca. 10 cm. (Photograph credit: R. O'Donnell)

Figure 9. Map of fishing vessel traffic between the island of Newfoundland and the Maritime Provinces of Canada. Data obtained for 1051 vessels between 2003-2012 from the VUE database of Fisheries and Oceans Canada, for vessels > 35 feet long equipped with a Vessel Monitoring System. The map shows typical vessel tracks for a subset of vessels represented by different colours (n=13). NL = island of Newfoundland, PE = Prince Edward Island, NS = Nova Scotia, and NB = New Brunswick. (Map credit: T. Wells, M. Hurley, and J. Sheppard).

3.0 RISK ANALYSIS OF INVASION

3.1 HOW LONG HAVE *BOTRYLLUS SCHLOSSERI* AND *BOTRYLLOIDES VIOLACEUS* BEEN IN NEWFOUNDLAND?

It is not known how long *Botryllus schlosseri* and *Botrylloides violaceus* have been in Newfoundland. *B. schlosseri* was first observed in 1945 (U.S. Navy 1951), again in 1975 (Hooper 1975) and by our research group in 2006 (Callahan et al. 2010). We hypothesize that *B. violaceus* has been in Newfoundland for a shorter time than *B. schlosseri*, because of its low genetic diversity of COI (Callahan et al. 2010), and because it has only been found in a single harbour as of April 2013.

3.2 WHAT IS THE LIKELY INVASION HABITAT IN NEWFOUNDLAND?

Due to the transport of NIA by anthropogenic vectors, man-made structures in harbours are the most common invasion habitat in Newfoundland. These structures include fixed wharves, floating docks, submerged chains, lines, ropes, and vessel hulls (Figs. 8 and 10). *Botryllus schlosseri* and *Botrylloides violaceus* are less common on naturally occurring substrates, but can be found on rocks, boulders, kelp, and eelgrass in some harbours. It is hypothesized that the anthropogenic creation of artificial substrates in harbours and aquaculture sites is a major factor in facilitating species invasion globally (Glasby et al. 2007).

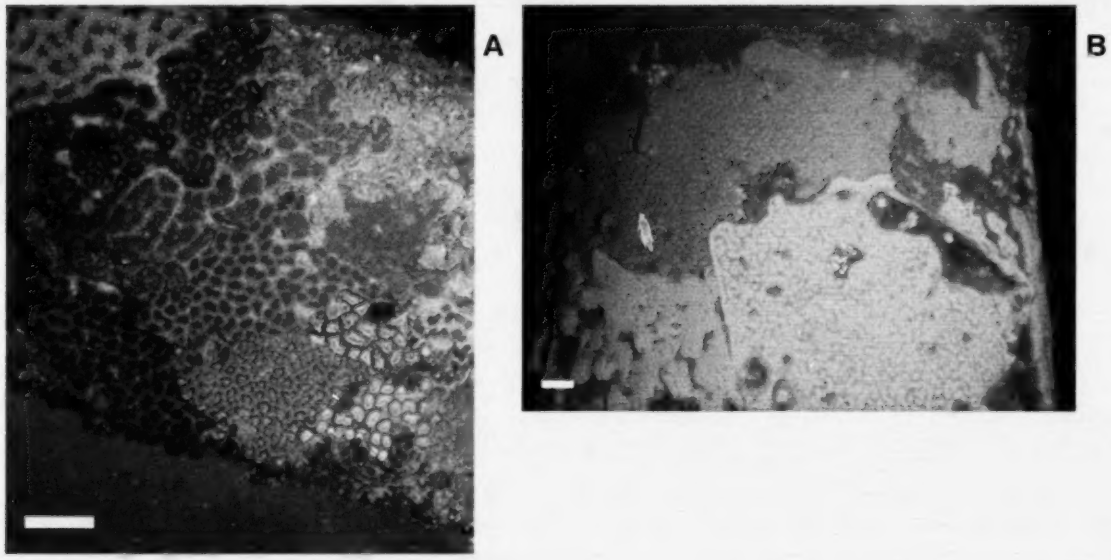

Figure 10. Common anthropogenic substrates fouled by *Botryllus schlosseri* and *Botrylloides violaceus*. (A) Colonies of *B. schlosseri* of about seven colour morphotypes fouling the edge of a floating dock in Foxtrap, Newfoundland. (Photo credit: R. O'Donnell) (B) Colonies of *B. violaceus* of four colour morphotypes fouling fixed wharf pilings in Belleoram, Newfoundland. Scale bars in A and B = ca. 2 cm. (Photo credit: R. O'Donnell)

3.3 WHAT IS THE PRESENT EXTENT OF *BOTRYLLOIDES VIOLACEUS* IN BELLEORAM HARBOUR?

In Newfoundland, *Botrylloides violaceus* is restricted to outer Belleoram harbour. However, its extent in Belleoram has increased since first discovered in 2007. Originally restricted to pilings supporting the inner half of the existing, fixed public wharf and the hulls of several small vessels, it now covers all of this wharf, an attached floating dock, a new extension to the fixed wharf built in 2009, floating docks and rocks along the inner harbour near the road and breakwater, and large boulders along the entire length of the outer breakwater. It was also found in the fall of 2012 heavily fouling the hull of a finfish-aquaculture, food barge. It has not been found fouling aquaculture cages.

3.4 WHAT IS THE PRESENT EXTENT OF *BOTRYLLUS SCHLOSSERI* IN NEWFOUNDLAND?

Botryllus schlosseri presently (June 2013) has been found in 23 harbours, primarily along the south coast of Newfoundland (Fig. 5). In the fall of 2011, *B. schlosseri* was discovered for the first time in Conception Bay, on the northeast coast of the Avalon Peninsula.

3.5 WILL *BOTRYLLUS SCHLOSSERI* AND *BOTRYLLOIDES VIOLACEUS* DIE OUT NATURALLY?

Once established, it is unlikely that either of these two species will die out naturally. However, *B. schlosseri* was present in Bonne Bay and St. Paul's Inlet, Newfoundland, in the early 1970s (Hooper 1975), but is now absent in those locations (B. Hooper, pers. comm.). Elsewhere in the Atlantic Ocean, these two species seem to be members of a successional sequence of invasive species, often replaced by other, more competitively dominant invaders (Gittenberger and Moons 2011). It is thought that colonial ascidians are pioneering invaders, followed in order by solitary ascidians, arborescent byrozoans, and finally encrusting bryozoans, which are members of the fouling climax community (Dijkstra et al. 2007). Thus, although NIA may disappear from a location, they are likely to be replaced by a more competitive invader. Where *B. schlosseri* and *B. violaceus* occur together, *B. violaceus* is generally the superior competitor (Dijkstra et al. 2007, Gittenberger and Moons 2011).

3.6 WHAT IS THE POSSIBILITY FOR REGIONAL SPREAD AROUND THE ISLAND?

Based upon the wide spatial extent of *Botryllus schlosseri* and the superior competitiveness of *Botrylloides violaceus*, we conclude that the possibility for spread of both species around the island of Newfoundland is high. Our research provides much evidence of the reasons for establishment of *B. schlosseri* in Newfoundland. First, we found this species to have high overwintering survival at temperatures below 5°C, enabling it to persist in the cold coastal waters of Newfoundland (Lowen et al. in prep.). Secondly, early reproduction allows for 8 or more generations during each growing season. This life-history adaptation is advantageous when the growing season is time constrained by low temperature (Lowen et al. in prep). Thirdly, the relative absence of predators and competitors raises the probability of establishment when spread to new environments. Lastly, vessel traffic, and the movement of fishing and aquaculture equipment, and aquaculture product, all significant in Newfoundland, provides vectors which may accelerate regional spread.

3.7 HOW LIKELY IS THE INVASION OF MUSSEL FARMS?

The recent risk assessment models of Therriault and Herborg (2008) indicate most of the south and west coasts of the island of Newfoundland to be at high risk for the establishment of *Botryllus schlosseri*. Furthermore, the southwest coast, between Gros Morne National Park and Port-aux-Basques, is at risk for the establishment of *Botrylloides violaceus* (Therriault and Herborg 2008). This places mussel aquaculture farms on the south coast at high risk for the establishment of both species. There are presently mussel farms in Placentia and Connaigre bays. In addition, given the high

overwintering survival and short generation times of these NIA, mussel aquaculture farms along the northeast coast of the island may come under threat of invasion in the future, including farms in Notre Dame Bay and Green Bay. For example, in the fall of 2011, we discovered *B. schlosseri* on the northeast coast of the Avalon Peninsula in Foxtrap harbour, Conception Bay.

3.8 HOW LIKELY IS THE INVASION OF SALMON FARMS?

Finfish farms along the south coast of the island of Newfoundland are also at risk for the establishment of *Botryllus schlosseri* and *Botrylloides violaceus*. Finfish aquaculture is taking place near Belleoram, Fortune Bay, with many large sea cages in the region. Since 2007 we have documented the spread of *B. violaceus* within Belleoram harbour, finding in the fall of 2012 that rocks forming the outside of the breakwater were covered by colonies. At the same time, SCUBA divers found the hull of a food barge to be infested with *B. violaceus*. Since the barge transports food from the infested harbour to the sea cages, they may be at risk of colonisation by *B. violaceus*.

3.9 WHAT HAS BEEN THE ECONOMIC IMPACT ELSEWHERE IN THE REGION, AND IN CANADA?

Colonial ascidians were judged to have a high to very high impact on shellfish aquaculture and a moderate impact on finfish aquaculture in an online survey of Canadian experts (Therriault and Herborg 2008). In general, *Botryllus schlosseri* was judged to be of lower impact than *Botrylloides violaceus,* and non-indigenous, solitary ascidian species. However, there have been no cost estimates of the impact of colonial ascidians on the aquaculture industry. Globally, there have been estimates of the direct costs of biofouling from all sources to the aquaculture industry (Fitridge et al. 2012). These estimates are 20-30% of market value for oysters and scallops. We were not able to find a similar estimate for mussels. *Botryllus schlosseri* was discovered in PEI in 2001, while *Botrylloides violaceus* was reported in 2002 (Locke et al. 2007). Although both species have colonized some bivalve aquaculture farms, they have been less of a problem for the PEI shellfish aquaculture industry than have solitary species (Bakker et al. 2011). Although the PEI mussel aquaculture industry generated ca. $48,000,000 of revenue in 2005 (Bakker et al. 2011), there has been no estimate of the cost of fouling by solitary or colonial ascidians. If we take the global estimate of the cost of biofouling for the bivalve aquaculture industry of 25% of market value (Colautti et al. 2006, Fitridge et al. 2012), this would mean that the cost of biofouling of mussel farms in PEI is ca. $12,000,000 per annum, based upon the 2005 market value of $48,000,000. This can be compared to the cost of the oyster thief to the oyster aquaculture industry in Canada of $2,500,000 per annum, the cost of the European green crab to the capture fisheries for lobster and rock crab in the southern Gulf of St. Lawrence and Newfoundland of $44,000,000 per annum, and the cost of the sea lamprey to the commercial and sport fishing industries in the Great Lakes of $138,000,000 per annum (Colautti et al. 2006). The costs of ascidian biofouling in PEI and Nova Scotia have made some bivalve farms economically unsustainable (Carver et al. 2006). One must remember however, that most of the costs to the PEI industry are due primarily to solitary ascidian species, and less so to *B. schlosseri* and *B. violaceus*.

As of 2012, there has been limited economic impact of fouling by *Botryllus schlosseri* and *Botrylloides violaceus* on the aquaculture industry in Newfoundland. The only impact so far was the prohibition of transport of mussels from Placentia Bay to processing plants in 2007, due to the potential for biofouling of product by *B. schlosseri*. This affected the economic return of a single mussel grower in Placentia Bay that year. Cleaning protocols have been put in place and the export of mussels from Placentia Bay has resumed.

4.0 FEASIBILITY OF ERADICATION

4.1 IMPORTANCE OF EARLY DETECTION AND LOCAL SCIENCE

One of the most important factors in determining the success of eradication is early warning of an invasion shortly after it occurs (Meyers et al. 2000, Bax et al. 2001, vander Zanden et al. 2011, Pluess et al. 2012). The key factor appears to be the spatial extent of the target population, with smaller populations being more easily exterminated. In addition, local scientific information is required to inform eradication decision making (Bax et al. 2001). There are several important scientific questions upon which good management depends. What is the annual life cycle of the invader? When does it spawn? When are larvae released? What is its reproductive potential? Given this biological information, what is the best method for eradication? When is the best time for carrying out eradication? All of these questions are optimally answered only with the highest quality, site-specific, scientific information available. This research process, if carried out thoroughly, can take years to accomplish (Bax et al. 2001).

4.1.1 Surveys

There are many different types of surveys for the detection of marine invasive species (Campbell et al. 2007). Although each has its own specific objectives, they can be broadly classified into quantitative and qualitative approaches. Most importantly, the objectives of a given survey must be clearly defined before choosing a particular approach. We have summarized the following five protocols from a comprehensive review by Campbell et al. (2007).

The oldest, and most frequently used quantitative approach, is the Hewitt and Martin protocol, developed at the Centre for Research on Introduced Marine Pests (CRIMP). The CRIMP protocol aims to detect and determine the distributions of native, cryptogenic, and introduced species, and to determine invasion pathways and vectors. They are based upon well-founded statistical theory (i.e., the Poisson distribution) and are designed to maximize the probability of detecting rare, introduced species. The CRIMP protocol requires a relatively large team, including SCUBA divers and surface vessels. Thus, it is relatively time consuming and costly.

Perhaps the most frequently used qualitative protocol is the Rapid Assessment Survey (RAS) (Arenas et al. 2006). The objective of the RAS protocol is to detect the presence of native, cryptogenic, and introduced species. It is based upon collecting samples that are within arm's reach or snorkeling depth, such as from floating docks, intertidal rocks, etc. Thus, SCUBA divers and boats are not required. RAS can be difficult to

standardize, but can be normalized based upon a pre-determined maximum search area or time (Arenas et al. 2006). RAS can only be internally compared if the same taxonomic expertise is available for all surveys. While the presence of species is statistically robust with RAS, the absence of a given species is not. In general, RAS are less costly and time consuming than the CRIMP protocol.

Staff at the Bernice P. Bishop Museum (BPBM) have developed a protocol that utilizes both quantitative and qualitative methods. The BPBM protocol has the same objectives as the CRIMP protocol. Quadrat samples, sediment cores, and qualitative SCUBA diver transects are all employed. Teams are generally small and thus field costs are minimal.

The Chilean Aquaculture Survey (CAS) is a qualitative protocol that was developed to detect introduced and escaped species of importance to aquaculture. It uses a line-transect SCUBA diver survey similar to that of the BPBM protocol. The surveys are designed following a distance sampling statistical model and the raw data are collected on video for later analysis. Limitations of this protocol are primarily due to observer bias. The teams are relatively small and costs low.

Passive sampling (PS), using settlement plates, is becoming an increasingly popular quantitative protocol for determining the presence of NIA. The objectives of this technique are broad, including determination of the presence of introduced species, species distributions, vector patterns, early warning, and timing and rates of larval recruitment (see review by Ma 2012). It is generally recommended to deploy PVC plates or petri dishes within a few meters of the water surface (Sephton et al. 2011). Plates are later retrieved, and the undersides examined microscopically for the presence of newly settled oozooids, or juvenile or adult colonies, depending upon the time period of deployment (Ramsay et al. 2008). If the goal of the exercise is to determine the presence of NIA, then the plates can be deployed for the full period of the growing season. If the goal is to determine the timing of larval settlement, then the plates are deployed, retrieved, and new plates redeployed on a shorter time scale, such as bi-weekly (Ma 2012). Since some larvae do not prefer to settle on clean surfaces, the plates should be pre-soaked in filtered sea water (Ma 2012). It is advisable to combine PS and RAS to spot-check PS results (G. Lambert, pers. comm.). PS require a small team and are thus cost effective. They have the power to collect time-integrated samples, which the other protocols above do not (Bishop and Hutchings 2011). Statistical models used for PS depend upon survey objectives, but have included Poisson, distance-from-source, and random-stratified models. The PS protocol has been adopted by the Aquatic Invasive Species Atlantic Zone Monitoring Program (AIS-AZMP), begun by DFO in 2006 (Sephton et al. 2011).

4.1.2 Species-specific genetic markers to detect fragments or larvae in plankton tows

Species-specific genetic markers, such as COI sequence information and quantitative polymerase chain reaction (QPCR) assays, are now available for many of the indigenous and non-indigenous ascidians present in Canadian coastal waters (Callahan et al. 2010, Bock et al. 2011, 2012, Willis et al. 2011). The base-pair sequence of the

entire nuclear genome has been determined for *Ciona intestinalis* (Dehal et al. 2002) and *C. savignyi* (Small et al. 2007). *C. intestinalis* type B is present in eastern Canadian waters, including Newfoundland (Sargent et al. 2013). The nuclear genomes of several additional ascidian species, including *Botryllus schlosseri*, are currently being sequenced (Lemaire 2011). In addition, genomic resources, such as cDNA libraries and expressed sequence tags (ESTs), have been generated for several ascidian species, including *Botryllus schlosseri* (Gyoja et al. 2007, Oren et al. 2007, Gasparini and Shimeld 2011), and the mitochondrial genome has been sequenced for 5 ascidian species (Singh et al. 2009). We have recently determined the sequence of a segment of the COI mitochondrial gene of *Botryllus schlosseri* and *Botrylloides violaceus* from Newfoundland populations (Callahan et al. 2010, Applin et al. in prep.). Based on these sequences, TaqMan® QPCR assays have been developed and tested for *B. violaceus*, and are currently being developed for *B. schlosseri*, to enable the species-specific detection of a single larva or colony fragment contained within a bulk zooplankton tow (Applin et al. in prep). Ultimately, this type of TaqMan® QPCR assay can be used by laboratories to provide aquaculture site assessments and early warning of the presence of these NIA. A few groups around the world are already doing research on methods for placing multi-species arrays of gene probes onto ocean moorings to provide remote, real-time warning of the presence of individual species of prokaryotes and eukaryotes (Preston et al. 2011).

4.1.3 Site-specific information on the seasonal cycles of growth, reproduction, larval settlement, and temperature

Detailed studies of the life-history and distribution of an organism are required to establish where and when to target eradication efforts. It is especially important to remove a NIA before it reproduces, spreads, and increases its population size. Our research has shown that local-temperature time series can be used to predict the seasonal cycles of asexual growth, sexual reproduction, and larval settlement in *Botryllus schlosseri* and *Botrylloides violaceus* (see Section 2.4.1). Thus, only easily obtained temperature data is required to establish when to carry out eradication measures for these species in Newfoundland. To prevent the re-establishment of populations by larval settlement, mitigation attempts will be most effective if undertaken before sexual reproduction begins. Any eradication effort involving physical removal of colonies must not create and release fragments of colonies back into the environment, as they can reattach and regenerate into new colonies, with unintended, counterproductive consequences.

4.2 METHODS

4.2.1 Global and regional

Many measures have been developed to attempt to limit the impact of ascidian biofouling on human societies and economies. Some of these measures have included attempts to eradicate NIA entirely from a harbour or aquaculture farm. However, *Botryllus schlosseri* and *Botrylloides violaceus* attach to many artificial and natural substrates, and readily occupy dark cracks and crevices under wharves or among rocks. Combined with a life cycle that facilitates rapid regeneration and establishment of

populations, eradication is not easy. We will summarize the results of eradication attempts below (see Sections 4.2.3 and 4.2.4).

Experimental methods to eradicate invasive ascidians have included mechanical, chemical, and biological techniques. Mechanical methods include manual removal by SCUBA divers (Switzer et al. 2011), underwater vacuum suction, suffocation using plastic sheeting (encapsulation) (Coutts and Forrest 2007), high pressure water or steam jets (Arens et al. 2011, Paetzold et al. 2012), aeration (Bullard et al. 2010), ultrasound (Guo et al. 2011), and light (Grosberg 1988, Bingham and Reitzel 2000). With respect to the fouling of vessels and floating docks by invasive ascidians, the simplest method is to lift the structures ashore and air dry for at least 48 hours (Hopkins and Forrest 2008). The organisms should then be scraped from the hull taking care not to reintroduce fragments into the ocean. These mechanical methods to eradicate populations of invasive ascidians are often time consuming, expensive, and unsuccessful. Some, such as high pressure water, may, by encouraging fragmentation, exacerbate the problem (Paetzold et al. 2012). Others have consequences that may be undesirable, such as destroying the entire macrobenthic community (e.g., encapsulation) (Kleeman 2009), or reducing the yield of farmed shellfish (LeBlanc et al. 2007).

Chemical treatment is another option for removing invasive ascidians (Forrest et al. 2007). Chemical treatment trials at mussel cultivation sites have included acidic (acetic or citric acid) or alkali (calcium hydroxide, sodium hydroxide, sodium hypochlorite) washes of mussel cultivation ropes, and the soaking of mussel ropes in freshwater (Forrest et al. 2007, Denny 2008, Kleeman 2009, Page et al. 2011, Switzer et al. 2011). At the recommended concentration and treatment duration, these treatments all kill invasive ascidians (see review by Carver et al. 2006, Forrest et al. 2007). Unfortunately, they require large quantities of the active agent to work effectively (Kleeman 2009), and often kill or reduce the growth of other macrobenthos, including farmed shellfish (LeBlanc et al. 2007). In combination with a significant additional investment in labour and husbandry, it is difficult to scale up the chemical treatment options in an economically and environmentally sustainable manner. Recently, several allelochemicals have been screened for anti-metamorphic activity in the solitary ascidian *Ciona savignyi* (Cahill et al. 2012). Three compounds have shown promising anti-ascidian properties.

Specially-formulated paints have been used to control fouling organisms on ship hulls and underwater structures for decades. Some of these coatings (e.g., tributyl tin, TBT), have had serious unintended consequences, such as disrupting marine food webs, compromising ecosystem health. However, new, primarily copper-based products have been developed to replace TBT. Biocides have yet to be developed that do not affect the physiological functions of exposed, non-target, organisms (Cima et al. 2008, Guardiola et al. 2012). Food-grade oil (i.e., vegetable shortening) has also been used in PEI for the inhibition of settlement of NIA (Bakker et al. 2011).

Other experimental treatments have tested the introduction of predators to control the rate of spread of invasive ascidians in shellfish cultivation sites (Epelbaum et al. 2009b). Naturally occurring predators of *Botryllus schlosseri* and *Botrylloides violaceus* include chitons, flatworms, sea urchins, sea stars, snails, limpets, nudibranchs, and crabs (Osman and Whitlach 2004, Nydam and Stachowicz 2007, Grey 2010, Switzer et al. 2011). These predators can be housed in cages or trays alongside the cultivated bivalves. The green sea urchin, *Strongylocentrotus droebachiensis*, has been shown to be a particularly effective predator of *B. schlosseri* and *B. violaceus* in the laboratory (Epelbaum et al. 2009b), while chitons have heavily grazed colonial ascidians in the field (Nydam and Stachowicz 2007). However, field studies have shown that *S. droebachiensis* prefers to eat native ascidians rather than *B. violaceus* (Simoncini and Miller 2007, Switzer et al. 2011), suggesting that the green sea urchin may not be useful to eradicate invasive populations of colonial ascidians.

4.2.2 Local

From 2008-2010, DFO worked with MUN to test various eradication and control methods on *Botrylloides violaceus* in Belleoram harbour, Newfoundland. Methods tested included wrapping wharf pilings and boat hulls in plastic (i.e., encapsulation) (Fig. 11), manual removal of colonies from wharf pilings and boat hulls by SCUBA divers, removing infected rocks manually followed by benthic vacuuming by SCUBA divers, and introducing caged sea urchins. Plastic wrapping materials were cheap and readily available, and the work was completed by a dive team in a relatively short time.

An obstacle to installing plastic during future eradication attempts in Belleoram will be the crib construction of the new wharf extension, which will be extremely difficult to encapsulate. In addition, the crib construction vastly increases the habitable surface area for NIA beneath the wharf, thereby potentially supporting a much larger population of *Botrylloides violaceus* than might other wharf designs. Our most recent survey in Belleoram, in 2012, revealed that the new wharf structure is already heavily fouled by *B. violaceus*, only three years after its construction. All of these costs and benefits must be weighed carefully before implementing encapsulation.

In the fall of 2011, *Botryllus schlosseri* was discovered in abundance on several floating docks in Foxtrap harbour, Conception Bay. The docks were lifted from the water using a mobile crane in December of 2011. They were refloated in May 2012.

4.2.3 Success rate and factors global and regional

The single most important factor in successfully containing or eradicating NIA is the size (i.e., spatial extent) of the invasive population (Pluess et al. 2012). However, since population size generally depends upon time since introduction, it is most important to detect the presence of a NIA early, when the population size is still small (vander Zanden et al. 2011). While eradication requires 100% mortality of the target invasive species, control may be effective at some mortality rate < 100%.

There have been few published tests of methods for eradicating or controlling *Botryllus schlosseri* and *Botrylloides violaceus*. High pressure water has effectively eradicated *B.*

schlosseri and *B. violaceus* from mussel socks (Arens et al. 2011, Paetzold et al. 2012). However, the reduction was only temporary, and recolonization of clean surfaces took place rapidly, perhaps in part due to reattachment of fragments generated by the spraying process (Paetzold et al. 2012). In addition, repeated spraying within a single growing season resulted in ca. 30% decrease of mussel abundance and biomass on the socks (Paetzold et al. 2012). Thus, the use of high pressure water to reduce fouling by colonial NIA in the PEI mussel industry is not recommended (Paetzold et al. 2012). Importantly, Paetzold et al. (2012) found that the seasonal timing of high pressure spraying was more important to the success of this method than was the frequency of spraying, and that spraying just before harvest was most beneficial to growers.

Aeration by bubble streams has been found to reduce fouling of PVC plates by these two colonial ascidian species by almost 100% (Bullard et al. 2010). Aeration is relatively environmentally friendly and potentially could be deployed deep within the wharf substructure. Success of aeration depends upon a continuous stream of bubbles, and direct exposure of the fouled surface to the bubble stream (Bullard et al. 2010). Aeration has yet to be tested at the scale of entire wharfs or on aquaculture farms. It is possible that aeration of fouled mussel lines would interfere with mussel feeding and metabolism if carried out for an extended period of time (i.e. > 1 wk).

A 4% acetic acid solution (vinegar) was 100% effective in eradicating *Botryllus schlosseri* after only 1 min of exposure (Forrest et al. 2007). A 2% solution did not eradicate *B. schlosseri* even after 4 min of exposure. Mussel survival was high in the 4% acetic acid treatments, and was independent of exposure time (Forrest et al. 2007). However, the mussels must be shaken before dipping to induce valve closure before immersion. Vickerson et al. (2011) found that dipping mussel seed in a brine solution before transport was most effective in controlling colonial ascidians, with little subsequent impact on mussel growth.

In laboratory trials, the sea urchins, *Strongylocentrotus droebachiensis* and *S. franciscanus,* reduced fouling by *Botrylloides violaceus* by 40-90%, and by *Botryllus schlosseri* by ca. 70-80% (Epelbaum et al. 2009b). Mortality of both colonial ascidians was ca. 100% due to predation by the sea star *Dermasterias imbricata*. A variety of crab and mollusc species caused very little mortality of *B. schlosseri* and *B. violaceus*. Mortality rate depended upon alternative prey availability however, so that mortality due to sea urchins and sea stars in the field may be less than these laboratory-determined rates (Simoncini and Miller 2007, Epelbaum et al. 2009b). Colonies that have been preyed upon show characteristic morphologies (Epelbaum et al. 2009b). Of the thousands of colonies we observed during our field work in Belleoram and Arnold's Cove harbours, we did not see any colonies showing evidence of predation by sea urchins or sea stars. In a field trial on floating docks, snail and flatworm predators did not have a significant impact on the abundance of *B. violaceus* (Grey 2010), and *S. droebachiensis* preferred native ascidians to *B. violaceus* in the Gulf of Maine (Simoncini and Miller 2007). However, this question remains outstanding, since previous studies had found large negative effects of chiton and snail predators on the abundance of *B. schlosseri* and *B. violaceus* (Nydam and Stachowicz 2007).

Regardless of this uncertainty, it is clear that no predator is likely to completely eliminate NIA. Therefore, the use of predators may be useful only as a control measure.

Of the many methods tested for eradicating solitary ascidians from mussel lines in PEI, only high pressure water, immersion in acetic acid (vinegar) and coating buoys and lines in vegetable shortening has been effective. High pressure water is nearly 100% effective in removing solitary ascidians from mussel lines, but requires running the lines through a vessel-mounted spraying cabinet, a process which is capital and labour intensive. Unwanted side-effects of spraying may be dislodging mussels and releasing ascidian gametes, larvae, and fragments which may contribute to new recruitment. Immersion in a vinegar bath is less effective than high pressure water, and requires a surface soak of several minutes (LeBlanc et al. 2007). Also, a 30 s dip in 5% acetic acid resulted in a reduction of mussel weight of ca. 70%, 7 months later. Thus, acetic acid has not been used as yet in farm applications. Coating aquaculture equipment in vegetable shortening reduces fouling by solitary ascidians by ca. 30% on spat collectors and 95% on buoys (Bakker et al. 2011). However, applying the oil is labour intensive, and mussel biomass may be reduced by ca. 25% in comparison to untreated lines (Bakker et al. 2011). Thus, shortening is not yet being used routinely by farmers in PEI.

Freshwater dips were found to be moderately effective in reducing fouling by the invasive colonial ascidian *Didemnum vexillum,* depending upon exposure time (74-87% mortality) (Denny 2008). However, at acetic acid concentrations and exposure times that did not harm the mussels, *D. vexillum* experienced ca. 80% mortality. Depending upon vinegar concentration and exposure time, mussel mortality ranged from 1-87% (Denny 2008). Dipping fouled seed mussels in a 0.5% sodium hypochlorite (bleach) solution for 2 min eradicated essentially 100% of the *D. vexillum* while having little effect on the mussels. Lime, sodium metasilicate, and sodium hydroxide were ineffective in eradicating *D. vexillum* (Denny 2008). However, Switzer et al. (2011) found that mechanical removal or an application of 4% hydrated lime reduced the abundance of *D. vexillum* by ca. 90%. But in this case, the open space created by the removal of *D. vexillum* was rapidly occupied by *Botryllus schlosseri* and *Botrylloides violaceus* (Switzer et al. 2011).

Encapsulation of vessels in plastic sheeting has been effective in eradicating *Didemnum vexillum* (Coutts and Forrest 2007). However, the development of anoxic conditions requires considerable time, making this approach more useful for fixed structures, like wharves, and seasonally inactive vessels.

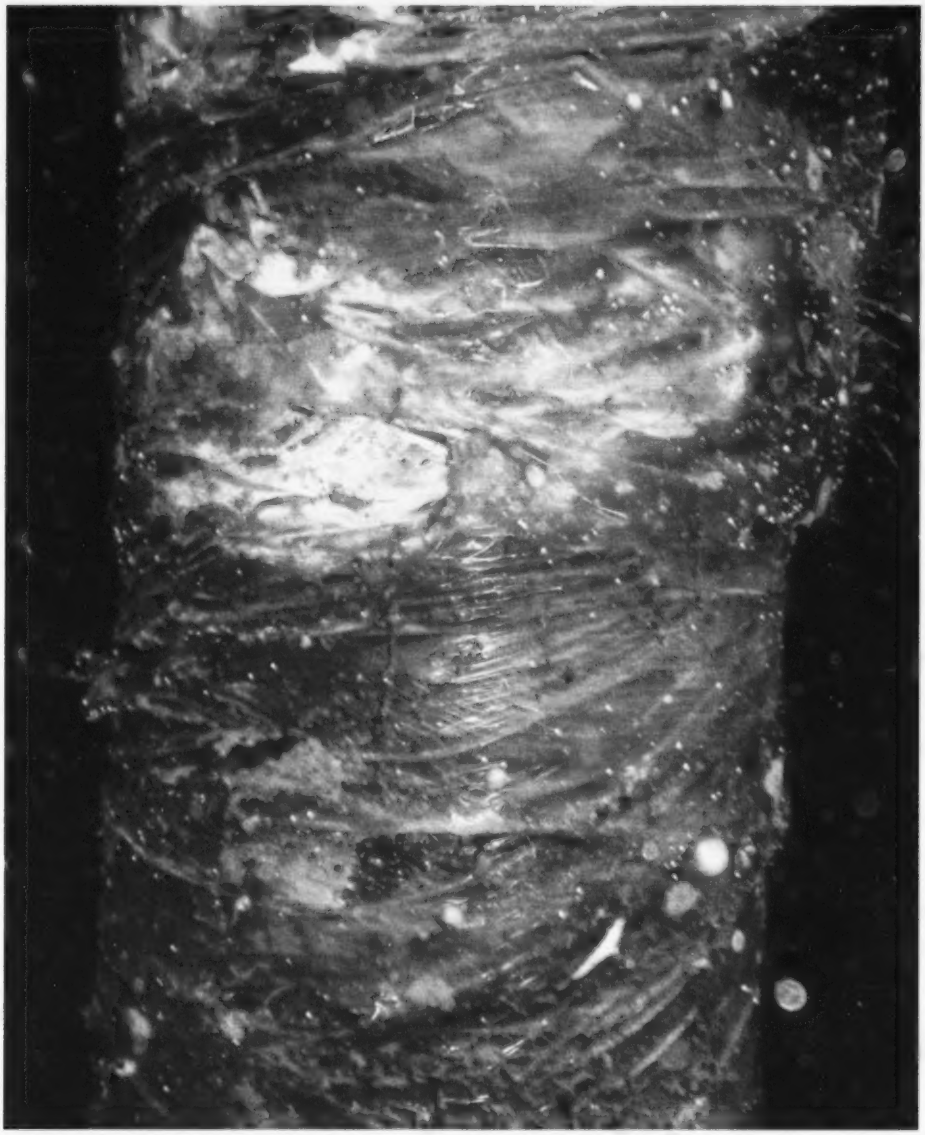

Figure 11. Encapsulation of a fixed wharf piling in Belleoram, Newfoundland, with sheets of plastic. Scale bar = ca. 2 cm. (Photograph credit: R. O'Donnell)

Direct spraying of concentrated acetic acid onto colonies of *Eudistoma elongatum* at low tide resulted in nearly 100% mortality (Page et al. 2011). Various alkalis and a heat treatment were ineffective. Interestingly, the addition of acid and alkali-stable surfactants to the chemical solutions increased tunic permeability to the toxicants, increasing their effectiveness (Page et al. 2011).

Relatively low, environmental concentrations of the allelochemicals radicicol, polygodial, and ubiquinone-10, have been found to reduce viable larvae of *Ciona savignyi* by ca. 100% in the laboratory (Cahill et al. 2012). However, subsequent tests have indicated

that radicicol has a negative effect on mussel growth and condition (Cahill et al. 2013). Allelochemicals against NIA have not yet been tested at commercial scales on an aquaculture farm.

4.2.4 Success rate and factors at local scales

If we define effective eradication as absence for at least two-times the longest generation time of the invader (i.e. > 19 months for *Botryllus schlosseri* in Newfoundland), none of the eradication trials we have carried out in Newfoundland has been successful. The initial results of our encapsulation trial in Belleoram harbour were promising. Following encapsulation on 16 March 2008, the first post-treatment assessment three months later (June 17, 2008) showed only 2% cover by small colonies of *Botrylloides violaceus* on one vessel. These colonies were promptly removed. All fouling organisms on wharf pilings were dead and rotting. No new growth of NIA was detected in the area. Seven months after encapsulation (October 7, 2008), we detected substantial re-growth of *B. violaceus* on much of the encapsulated bottom substrate and one of the encapsulated vessels. A third visit to the site eight months after encapsulation (October 29, 2008), showed some re-growth on wharf pilings and further growth on bottom substrate.

A second eradication attempt was made in Belleoram on 27 March 2009. The first follow-up assessment was done two months later, on 27 May 2009, when two small patches of *Botryllus violaceus* were found and removed. The next assessment was delayed until March 2010 by new wharf construction. Small colonies of *Botrylloides violaceus* were growing on the plastic used to cover the bottom substrate. However, most of the re-growth was observed on the plastic used to cover the wharf pilings and substrate underneath the wharf (Fig. 12) (Coutts and Forrest 2007). *B. violaceus* had also begun colonizing the new section of wharf, large rocks under the wharf, and several vessels.

Given the annual occurrence of sexual reproduction and dispersal in *Botrylloides violaceus*, encapsulation may be more effective if pilings are wrapped each year prior to sexual reproduction. The most likely sources of larvae and fragments for recolonization are the ballast rocks and substructure surfaces beneath permanent wharves, as well as untreated vessel hulls. As can be seen in Fig. 13, NIA are able to colonize deep within the sub-wharf environment. Eradication in this case is improbable. With abundant populations deep within wharf structures and on the hulls of numerous coastal vessels, it is difficult to eliminate all sources of re-colonization.
Removal of floating docks from Foxtrap harbour resulted in reducing the abundance of *Botryllus schlosseri* for the first growing season. However, several of the docks were re-colonized during the second growing season, likely from other populations within the harbour.

A trial predator addition experiment was run in Belleoram harbour. Sea urchins were placed in cages surrounding naturally occurring colonies of *Botrylloides violaceus* on wharf pilings. The trials were unsuccessful.

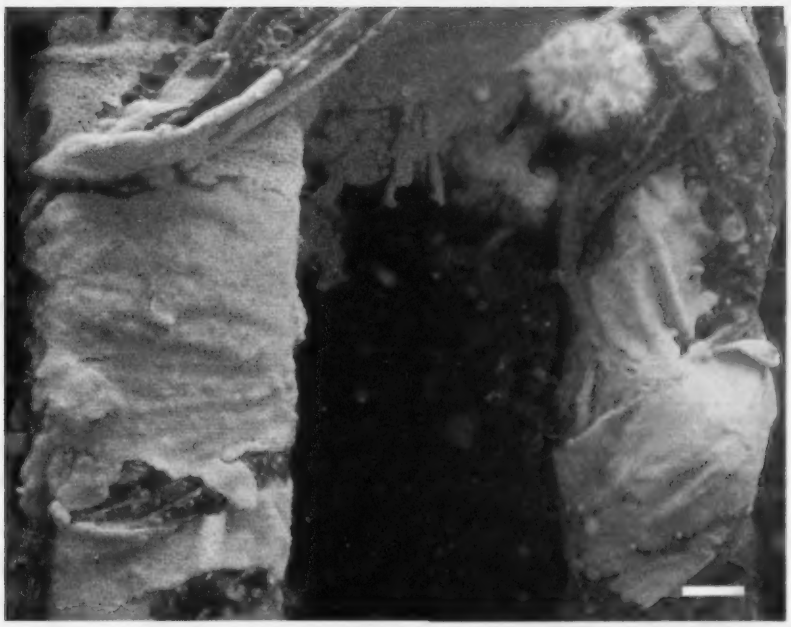

Figure 12. Plastic sheeting on wharf pilings covered by colonies of *Botrylloides violaceus* in Belleoram, Newfoundland. Scale bar = ca. 10 cm. (Photograph credit: R. O'Donnell)

Figure 13. Colonies of *Botrylloides violaceus* of two colour morphotypes living in small crevices deep within the fixed wharf substructure in Belleoram, Newfoundland. Scale bar = ca. 5 cm. (Photograph credit: R. O'Donnell)

4.2.5 Probability of successful eradication

Due to our inability to eradicate either *Botryllus schlosseri* or *Botrylloides violaceus* from two Newfoundland harbours, we judge the probability of successful eradication in Newfoundland to be low. In fact, neither of these species has ever been locally eradicated anywhere in the world. The likely reasons for our lack of success were that the size of the populations was too large and that there were too many sources of propagules for re-colonization. To increase the probability of successful eradication, effort should be made on early detection, public awareness, and the cleaning of fouled vessels and fixed harbour structures. Measures should be taken to prevent the spread to uncolonized harbours and aquaculture farms. This is more feasible than is eradication where these species have already become established.

4.2.6 Risks

Attempts to eradicate colonial, marine, benthic organisms have many associated risks. An organism like NIA that is a superior coloniser, but not a strong "successional" competitor, thrives in environments where habitat disturbance resets the successional process (Shea et al. 2004). This fundamental characteristic can lead to several unintended consequences of eradication measures, which are clearly major habitat disturbances. Techniques like encapsulation eliminate all fouling species, creating new space free of all macrobenthos. This newly opened space is the preferred habitat of colonisers like NIA. Thus, by creating cleared space, eradication may actually increase the rate of recruitment of NIA. Thus, eradication of NIA must be accompanied by other measures to reduce or eliminate sources of propagules for re-colonization, such as regulation of shipping vectors (Acosta and Forrest 2009, Lacoursière-Roussel et al. 2012).

All non-targeted measures (i.e., those that exterminate all macrobenthos), may have the unintended consequence of reducing native species diversity, destroying nursery habitat for desired species, or eliminating the commercially important species that the eradication measures were meant to protect (LeBlanc et al. 2007). Since NIA have not yet been found fouling aquaculture farms in Newfoundland, this risk is as yet minimal. Should fouling of commercially valuable product by NIA become a problem in Newfoundland in the future, the effect of any eradication treatment on the species-of-interest should be tested experimentally before large-scale application.

Finally, some eradication measures, such as high pressure water, may create fragments or release propagules, both of which result in increasing rates of invasive population growth rather than in eradication (Paetzold et al. 2012). For example, vessels to be cleaned of NIA must first be removed from the water, taking care to dispose of all cleared material on land (e.g. landfill), so as to avoid the release of colony fragments back into the ocean. Cleared material includes not only solid waste, but also water from the motor, bilge and sea chest.

5.0 FEASIBILITY OF CONTROL

5.1 THE IMPORTANCE OF MONITORING

Since the most important factor determining the success of eradication is the size of the invasive population, it is important to detect invasions shortly after they occur. There are at least three effective monitoring approaches which can be used either singly, or in combination.

5.1.1 Rapid Assessment Surveys (RAS)

RAS are a widely used technique to monitor for the presence of NIA (Campbell et al. 2007). However, if a large geographic area needs to be covered, RAS will be beyond the resources of any one agency or research group. Therefore, collaboration between various levels of government and between the public, academic, and private sectors will be required to monitor an area the size of the island of Newfoundland.

5.1.2 Gene probes

Gene probes are a more recent tool making early warning of an invasion more feasible (Lawrence et al. 2008). By definition, early warning must detect very small population sizes. Therefore, sensitive monitoring tools are required. Gene probes are sensitive, species-specific, and obviate the need for taxonomic expertise (Lawrence et al. 2008). Gene assays which can detect a single larva in a sample from a plankton net tow or pump are available for many NIA species (Turon et al. 2003, Stewart-Clarke et al. 2009, Callahan et al. 2010, Willis et al. 2011, Bock et al. 2011, 2012). Gene assays also have the potential for being incorporated into automated ocean moorings, making real-time detection of an invasion a possibility (Preston et al. 2011).

5.1.3 Passive surveys

Settlement plates are another tool which is valuable for monitoring the presence of NIA (Campbell et al. 2007). Settlement plates have many advantages, including that they are sensitive, and can be used to monitor an area over an extended time period without human intervention. In addition, because they are relatively cheap in materials and time, they can conceivably be deployed in many locations over a large geographic area. Finally, because they integrate over an extended time period, they have a higher probability of detecting transient events, such as a pulse of larval settlement.

5.2 IMPORTANCE OF PUBLIC VIGILANCE

Because the areas that may need to be monitored for the presence of NIA are generally large, it is important that a well-educated public is informed of the importance of remaining vigilant and of collaborating in the monitoring process (Locke and Hanson 2009). Public engagement in monitoring has the additional advantage of improving the probability of compliance with control measures (see Section 5.4).

5.3 VECTOR CONTROL

Since colonial NIA species have planktonic larvae with a very short free-swimming period, the primary life stage which is broadcast is the benthic colonial stage. Thus,

vectors for the spread of NIA are floating surfaces, such as vessel hulls, macroalgae blades, flotsam, jetsam, and mobile invertebrates, such as crabs and lobster (Bax et al. 2001, Acosta and Forrest 2009, Bernier et al. 2009, Lee and Chown 2009, Wilson et al. 2009). Additional vectors include other human activities, such as the transportation of bivalve seed, aquaculture product, and aquaculture equipment (Wilson et al. 2009). The feasibility of controlling most of the primary vectors is low, due to the free movement of vessel traffic, and to the natural sources of plant and animal vectors. However, voluntary and mandatory cleaning or control of regional and local coastal vessels has been successful in some cases in New Zealand and Australia (Pannell and Coutts 2007, Kleeman 2009). Important in these cases was vigorous and informative public education as to the risks and costs of invasive ascidian fouling, and the benefits of vector control (Kleeman 2009).

5.4 IMPORTANCE OF PUBLIC COMPLIANCE AND THE LACK OF POLITICAL WILL

Regulating ship traffic will be difficult. Historically, mariners have had relative freedom of the seas. Thus, there is little political will or power to regulate ship biosecurity and shipping lanes. The oceans are perhaps the last remaining commons on earth. However, public compliance with voluntary and mandatory regulation of hull cleanliness and shipping lanes will be of utmost importance if the control of invasive ascidians is to be feasible. This will require the political will to draft regulations, and an aggressive public education campaign to inform people that following regulations is important to their livelihoods and for the sustainable use of the oceans by future generations (Kleeman 2009). For example, Australia created the first regulations to control species invasions via hull fouling only in 2005, and only for internationally sailing pleasure vessels (Kleeman 2009). These regulations were made mandatory in 2006, and can be viewed at (http://www.daff.gov.au/biosecurity/avm/vessels). The heart of the regulation requires vessel operators to present evidence of having done at least one of the following; (1) cleaned the hull within one month before arrival, (2) applied anti-fouling paint within one year before arrival, (3) booked the vessel to be hauled out and cleaned within one week after arrival.

The potential for AIS to cause harm has been recognized by the International Maritime Organization (IMO), the Convention on Biological Diversity (CBD), several United Nations Environment Programme (UNEP) Regional Seas conventions (e.g., Barcelona Convention for the Protection of the Mediterranean Sea Against Pollution), the Asia-Pacific Economic Cooperation forum (APEC), and the Secretariat of the Pacific Region Environmental Program (SPREP). The guidelines adopted by the IMO in 2011 for 'The Control and Management of Ship's Biofouling to Minimize the Transfer of Invasive Aquatic Species' (Annex 26 Resolution MEPC.207(62) /24 Add.1), can be viewed at http://www.imo.org/blast/blastDataHelper.asp?data_id=30766&filename=207(62).pdf. These guidelines contain recommendations for practices which prevent the spread of AIS through biofouling of commercial vessels.

5.5 RISKS

It should be understood that it is impossible to eradicate invasive ascidians without some unintended consequences for the ocean environment. The best approach is to

realize this from the beginning, to consider possible unintended consequences, and to take all possible measures to minimize their occurrence (Bax et al. 2001).

6.0 RESPONSE OPTIONS

Response options fall along a continuum, from doing nothing, to attempting full eradication (Gust et al. 2008, Kleeman 2009). Responsible political entities (i.e., provinces, nations) must have in place a defined set of response options and a decision tree for moving among them. These decision trees can range from the relatively general (Bax et al. 2001), to the relatively specific and complex (Acosta and Forrest 2009). Biosecurity of the oceans must be a shared federal, provincial, and local responsibility. Next, we will define and describe the seven most common response options to NIA.

6.1 DO NOTHING

Since very few if any invasive ascidian populations have ever been eradicated (Williams and Grosholz 2008), and since NIA have not yet become an economic problem for Newfoundland aquaculture, doing nothing is a response that should be considered. However, because the probability of successful control of NIA ascidians depends upon the population size of the invader (Pleuss et al. 2012), we believe that doing nothing is not a responsible option in Newfoundland. In so doing we subscribe to the Precautionary Principle, which holds that the future spread and negative impact of invasive ascidians is difficult to predict. For example, global warming may extend the growing season of *Botryllus schlosseri* and *Botrylloides violaceus* in Newfoundland sufficiently that they may invade mussel farms in the future (Stachowicz et al. 2002b). In addition, *B. violaceus* is considered to have greater invasive potential than *B. schlosseri*. Thus, although *B. violaceus* is confined to a single harbour on the south coast of Newfoundland at present, this may not be true in the future. Therefore, responsible husbandry of the ocean environment encourages us to contain invasions when possible. The Canadian Action Plan to Address the Threat of Aquatic Invasive Species (DFO 2004) (available at http://www.dfo-mpo.gc.ca/science/enviro/ais-eae/plan/plan-eng.pdf) concludes that the prevention of introduction and spread is the most cost effective and successful response option to control invasive species. Where populations are already introduced the priority of the action plan changes to early detection and risk assessment. Doing nothing is not a responsible option.

6.2 LIVE WITH IT

Living with an invasive ascidian implies doing something rather than nothing. Living with the problem suggests turning the problem into a benefit. Some researchers believe that the invasion of a habitat actually increases total species diversity rather than decreases it (Craig 2010). In addition, many species of solitary and colonial ascidians are useful to human beings, as food, as a source of pharmaceuticals and nutriceuticals, and as model organisms for research on chordate evolution, stem cells, immunology, and cancer. Thus, it is possible to consider harvesting invasive ascidians for international food, chemical, and research markets. In fact, several ascidian species are grown on aquaculture farms in the orient and South America. It is possible that similar farms could be established in Newfoundland.

6.3 STAKEHOLDERS TAKE THE RESPONSIBILITY

This option depends upon stakeholders taking the responsibility to respond to the presence of NIA. While stakeholders will likely be the most highly motivated to respond, the requirements for doing so may be beyond their means. Eradication programs may need to cover a relatively large geographic area, and will likely be labour intensive, thus requiring relatively large sums of money to increase the chance of success. In one of the few marine examples of the eradication of an invader to date, one month, > 11 person-years of labour, and $2,000,000 (Aus), were required to exterminate a close relative of the zebra mussel from a single harbour in Australia (Bax et al. 2001). In Newfoundland, one of the leading stakeholder groups is likely to be the aquaculture industry. It is unlikely that this industry will have the economic means and ecological expertise to respond adequately to an invasion of NIA.

In addition, the stakeholder group may be relatively large, with diverse social and economic agendas. This may make a coordinated stakeholder response to an invasion of NIA relatively difficult to attain. Given the scope and difficultly of responding to invasive ascidians, a coordinated, focused approach is required.

6.4 MAP, MONITOR, AND INFORM

Once an invasion has been detected, it is important to map the extent of the population, to monitor its future spread, and to inform the public of the extent of the invasion (Locke and Hanson 2009). Mapping is generally done by visual means, either with a RAS, a visual survey by SCUBA divers, or a remote survey using a towed video camera (Campbell et al. 2007, Paravicini et al. 2010). The initial goal is to determine the spatial extent of the invasion. This can be done by surveying harbours far away from the initial detection point and then moving progressively closer, until a colonized harbour is encountered. Once the extent is defined, periodic monitoring is then conducted within and near the borders of the population to document its growth and spread. The public can help control the invasion if they are well informed. Protocols are developed for communication with the public. The information must be timely and accurate.

6.5 VECTOR CONTROL: VOLUNTARY

Voluntary vector control has been used to attempt control of invasive ascidian populations elsewhere around the world, with varying degrees of success (Gust et al. 2008, Kleeman 2009). With proper communication and public education, compliance generally increases. Since the primary vector for the spread of NIA is the fouling of vessel hulls, the control of ship vectors is of prime importance (Gust et al. 2008, Acosta and Forrest 2009, Wilson et al. 2009). If vessel owners can be shown that vector control benefits them directly, compliance increases.

6.6 VECTOR CONTROL: MANDATORY

If the infestation is severe, either in extent or economic impact, mandatory vector control may be required (Gust et al. 2008). The general public has come to accept mandatory vector control in the case of several agricultural pests (Meyers et al. 2000). Thus, given proper education and the economic imperative, mandatory vector control can be

accepted. Australia and New Zealand are the only countries that have some form of mandatory vector control for NIA (Gust et al. 2008, Kleeman 2009).

6.7 ERADICATE

Eradication is the most aggressive response to the presence of NIA. NIA are difficult to eradicate, as they are able to thrive in small cracks and crevices in the environment; places that are challenging to reach with most eradication measures. In addition, new recruits are small and difficult to detect by SCUBA divers. However, eradication that is only partially successful may still be one of the best control practices, as any decrease in the invading population size means less propagule pressure during the next spawning season. In many cases however, control is more feasible, less costly, and less damaging to the environment than eradication (Bax et al. 2001).

7.0 RECOMMENDATIONS

First, we recommend that provincial and federal governments develop and publish formal marine biosecurity rules and regulations, including a common-use, rapid-response protocol (Locke and Hanson 2009, Locke et al. 2011). The federal governments of Australia and New Zealand have already done so. The regulations of these countries could serve as a template for adaptation to the Canadian situation.

Secondly, we recommend that DFO, DFA, and Memorial University (MUN) colleagues collaborate in drafting an invasive species response framework for Newfoundland and Labrador. An obvious group to coordinate this collaboration is the Newfoundland and Labrador Aquatic Invasive Species Advisory Committee (NLAISAC). The NLAISAC includes members from industry, DFO, DFA, and MUN.

Before continuing with our specific recommendations for surveillance, eradication, and control, it is useful to review recent literature on the general problem of the response to and control of invasive species. This literature reminds us of the basic principles underlying reasoned, intelligent response to invasive species.

After a thorough review of the eradication literature on terrestrial and aquatic ecosystems, Meyers et al. (2000) identified six key requirements for a successful eradication program.

1. Resources must be sufficient to fund the program to its conclusion.

2. The lines of authority must be clear and must allow an individual or agency to take all necessary actions, even if public support for the program wanes.

3. The biology of the target organism must make it vulnerable to control measures. The term 'biology' includes dispersal ability, reproductive capacity, and life history.

4. Re-invasion must be prevented, from all potential sources. Vector control must be included in the cost-benefit analysis.

5. The pest must be detectable at very low population densities.

6. Environmentally damaging eradication might require some form of ecosystem restoration. Restoration must be included in the cost-benefit analysis.

Note that the first two requirements have nothing to do with the biology or ecology of the pest species. In general, we recommend that all six requirements are carefully and formally considered before planning and executing an eradication or control program for NIA in Newfoundland.

At about the same time as the Meyers et al. (2000) publication, Bax et al. (2001) presented seven necessary steps to be taken in any control program of a marine invasive species. Since the Bax et al. (2001) recommendations are specific to marine species, they are particularly pertinent to the case of NIA in Newfoundland. The seven recommendations are:

1. Establish the nature and magnitude of the problem. This step includes accurate taxonomic identification of the pest, linking the pest with particular environmental and human problems (including economic losses), and determining the spatial magnitude of the invasive population.

2. Set and clarify objectives. Stakeholders and decision makers need to determine what they want to accomplish. This is where the decision to eradicate or control must be made. This step will also define the criteria against which the success of the program will be measured.
3. Consider the full range of alternatives. These are presented in detail above (see Section 6). It is often advantageous to employ a suite of control options at the same time.

4. Determine the risks. This should be done carefully and thoroughly, from a precautionary point of view.

5. Minimize the risks. This can be done by detecting the invasion early, making the control program specific to the pest species, and experimenting on a small scale before scaling up.

6. Determine the cost-benefit ratio of the proposed control program. The support for control is dependent upon the magnitude of the problem × the probability of successful control, ÷ (the magnitude of adverse results × the probability of adverse results) + the cost of control. This relationship can be difficult to apply when the magnitude of the problem and risks cannot easily be assigned monetary values. In an appendix, Bax et al. (2001) present a potentially useful modification of this basic relationship for these problematic cases. {There are also other cost-benefit formulations available (Kleeman 2009). Colautti et al.

(2006) provide a broad example of an attempt to assign monetary values to many direct and indirect costs of invasive species in Canada.}

7. Monitor the performance of the program. This takes commitment and funding.

Locke and Hanson (2009) present a very detailed rapid-response framework to non-indigenous species in Canada that is partitioned into pre-invasion steps, which include general preparation, a detection phase, a demarcation phase, a containment phase, a risk assessment phase, an implementation phase, and a follow up phase, as well as post-invasion steps, which include all of the above phases except for general preparation. In essence, the Locke and Hanson (2009) framework combines the important requirements and recommendations from Meyers et al. (2000) and Bax et al. (2001).

Using the Bax et al. (2001) recommendations as a guideline, we next present our recommendations for the eradication and control of *Botryllus schlosseri* and *Botrylloides violaceus* in Newfoundland. Our strongest recommendation is to follow the hierarchy of prevention > eradication > control, to minimize the impact of NIA on Newfoundland and Labrador coastal waters. The best application of time and money is in prevention. This includes vector control. If an invasion cannot be prevented, the next best action is to eradicate it early, when the population is small. If eradication is not biologically or economically feasible, effective control of the invasive population can often yield significant benefits.

7.1 SURVEILLANCE

Surveillance is crucial for all steps of the response process, from the initial detection of the presence of a NIA, to determination of the spatial size of the invasive population, to post-eradication determination of effectiveness. We recommend a multifaceted surveillance approach for these various phases, including passive surveys using settlement plates, rapid assessment and/or SCUBA visual surveys, and genetic assays. We strongly recommend that funding be secured at the beginning of a response program for post-mitigation determination of its effectiveness. Without this crucial commitment, progress cannot be made in adaptive improvement and fine-tuning of response measures.

7.1.1 Passive survey

For the determination of the presence of a NIA in a location, as well as the spatial extent of an invasive population, we recommend a province-wide, passive survey using PVC settlement plates (Sephton et al. 2011). Passive surveys are among the least costly and most sensitive methods to detect the presence of NIA over a large geographic area (Campbell et al. 2007). We recommend deploying 10 × 10 cm gray, PVC plates on rope moorings at several locations within each harbour or aquaculture site. The plates should be roughened with medium grit sand paper, and pre-soaked in surface seawater for at least 14 d before deployment. This is to provide a microbial lawn on the plates which aids ascidian settlement. At least one plate should be attached to the line within 1 m of the surface, and a second plate within 1 m of the bottom. Additional plates can be

located at intermediate depths depending on the total water column depth at the site. The moorings can be anchored to the bottom with a building brick. The plates should be deployed in June, before the onset of sexual reproduction. They should be retrieved 4 months later, in October, when sexual reproduction has ceased. The plates should be photographed in the field, underwater, in an insulated container if possible. They should then be returned to the lab in seawater. In the laboratory, they can then be re-photographed under controlled light, followed by storage in 4 % formaldehyde in seawater, or in 95% ethanol if genetic analyses are to be done. The underside of the plates should be examined for the presence of oozooids and colonies, either directly with a microscope or hand magnifying lens, or by image analysis of the photographs.

7.1.2 Rapid assessment surveys

Once the presence of a NIA species is detected from the larval settlement plates, we recommend a RAS, accompanied by visual observations by SCUBA divers, to determine the small-scale spatial extent of the invasion. The rapid assessment and SCUBA surveys should cover all potential NIA habitats in a given harbour, including fixed wharf substructure, edges of floating docks, vessel hulls, and natural substrates, from the upper intertidal zone to the lower subtidal zone. Observations can be documented by underwater photography or videography. Spatial coordinates and depth should be recorded. At all times it is important that at least one member of the RAS and SCUBA teams is trained in the identification of NIA. Voucher specimens from each site should be preserved in 95% ethanol and archived.

7.1.3 Genetic assays

We recommend the use of species-specific, TaqMan® QPCR assays to confirm species identification. These assays, based upon COI sequence analyses of Newfoundland populations, are now available for the identification of *Botrylloides violaceus*, and are being developed for *Botryllus schlosseri* (Callahan et al. 2010, Applin et al. in prep.). Samples removed from those settlement plates fixed in ethanol, and from the rapid assessment and SCUBA surveys, can be screened with TaqMan® QPCR assays. In addition, if high sensitivity of detection is required, a plankton sample survey can be added to the settlement plate survey, and tested with TaqMan® QPCR assays, for the presence of NIA larvae or fragments in the water column (Applin et al. in prep.). A survey consisting of DNA templates from plankton samples, analyzed using TaqMan® QPCR assays, is the most sensitive surveillance technique, best applied to high risk locations where detection is required at sub-monthly time scales.

7.2 PUBLIC EDUCATION AND STAKEHOLDER CONSULTATION

Public education, and the resulting stakeholder consultation, is a crucial step to define and clarify the response objectives. The importance of community involvement in AIS monitoring cannot be over stated. Beginning with general awareness of the situation, building of partnerships and active involvement are all vital components of a community-based, early detection and monitoring programme. The AIS awareness campaign in Newfoundland is based on three R's: **Recognition** of the invasive species; **Removal** of invasive species from vessels, gear and the environment; and finally; **Report** the presence of new invasive species in the area. Reporting is done through a telephone

number, email and website that have been created for this purpose (http://www.nfl.dfo-mpo.gc.ca/nl/ais/aquatic-invasive-species). A website can have pictures and descriptions of the species as well as distribution maps. It also can provide information on what steps should be taken to prevent the spread of invasive species and how to remove them from vessels and gear. The website and other communication aids such as floating key chains, fact sheets, and information cards, can provide information on how to report invasive species.

Once general awareness has been heightened, a more targeted approach can be taken. Our initial partnerships were built with industries that are particularly affected by invasive species, such as aquaculture. We work closely with NAIA and DFA to monitor aquaculture sites and vessel traffic, and to plan and organize eradication trials. We also work closely with schools to provide information and curricula which not only inform the students, and their parents, about AIS, but also about scientific methods and the environment.

Other community groups to engage in early detection are recreational boaters and SCUBA divers. They are generally very aware of the marine environment and have a personal interest in keeping it free of invaders. It is becoming increasingly clear that biofouling of pleasure craft is one of the main vectors of regional spread of AIS in the marine environment.

We recommend early engagement of the local population once a new NIS is found in an area. This is usually most effectively conducted at the wharf, where information can be provided in a casual, hands-on atmosphere. Most Newfoundlanders are generally aware of NIS, so that when specific groups are approached they are receptive of information and willing to consider eradication activities and preventative measures.

We also recommend identifying and consulting stakeholder groups early in the response process. For NIS in Newfoundland, the stakeholders are the general public (i.e., towns and municipalities), the aquaculture and fishing industries, the appropriate labour unions, and the provincial and federal governments. All stakeholder ideas and concerns should be acknowledged, recorded, and considered for action. Frequent and serious stakeholder consultation is an important factor in increasing public compliance with voluntary and mandatory control measures.

7.3 PREVENTION

The best policy for minimizing negative impacts from NIA is to prevent their entrance into the province. Since the most likely vector for the transfer of NIA to Newfoundland is by sea going vessels, we recommend that steps are taken to ensure that the hulls of vessels entering Newfoundland harbours are free of NIA. This is particularly important because hull fouling is also the most likely source of propagules for re-colonization following eradication. While we cannot recommend the nature of the steps to be taken, or the level of regulations and their enforcement, perhaps something like those in place in Australia (see Section 5.4) might be appropriate. Similar regulations will be needed for intra-provincial vessel traffic of coastal and pleasure craft, as these smaller vessels

are the most likely vectors for regional spread around the island. At the very least, we recommend the immediate drafting of hull cleanliness standards, so that vessels known to be fouled by NIA are not permitted to enter non-colonized harbours unless documentation of recent cleaning is presented. This is particularly important for vessels moored in Belleoram harbour, which may be fouled by *Botrylloides violaceus*, which at this time is present only in Belleoram.

7.4 ERADICATION

If prevention is not possible, and the NIA is known to be present in a particular location, the decision to attempt eradication may be taken. Since any eradication measure by definition disrupts the target ecosystem, all possible measures should be taken to minimize risks of unintended consequences. Most important to minimize risk is to make the eradication program as specific as possible, both as to timing and protocol. Although none of our experimental extermination trials has been completely successful, the research we have conducted over the past three years enables us to recommend optimal times and methods of eradication.

7.4.1 Timing

We recommend that eradication be applied in May and June, when adult colonies are visible, but before seasonal sexual reproduction has begun. We believe eradication will be most effective, and unintended consequences minimized, if carried out before the onset of sexual reproduction. The appropriate timing can determined for any locale from local sea temperature. Sexual reproduction of *Botryllus schlosseri* begins at a temperature of ca. 11°C (Fig. 6). Sexual reproduction of *Botrylloides violaceus* may begin at slightly lower temperatures (Epelbaum et al. 2009a). Eradication should not be attempted during August-October when colonies are releasing larvae.

7.4.2 Method

We recommend removing colonized objects from the water if possible. Floating docks can be lifted to land in November and refloated in the spring. Vessels can be hauled out and scraped clean at any time of the year, making sure that no colony fragments are returned to the ocean. Rocks can be lifted with air bags and permanently disposed of on land.

If removal of the colonized object is not possible (e.g., a wharf piling), encapsulating it in plastic sheets is recommended. Sheeting should be installed in May or October, before or after the time of sexual reproduction. Suffocation can be accelerated by pumping freshwater, or freshwater + 0.5% bleach, inside the sheeting. The plastic should be left in place until all fouling organisms are exterminated. This may require 2-4 weeks. The sheeting should then be removed, and disposed on land. The sheeting should not be left in place longer than one month, as fouling organisms may settle on it, and be rafted elsewhere if the plastic is removed by waves or ice. As colonial ascidians living deep within the wharf substructure cannot be encapsulated, plastic may need to be applied several years in succession to reduce the local population below self-sustaining abundances. All colony fragments produced during the process of encapsulation must be removed to prevent re-colonization.

We know there are fouled vessels in many Newfoundland harbours that have NIA. Therefore, encapsulation will be most effective if coupled with a vessel decontamination program. Vessels should be inspected annually in May or June, prior to the reproductive season of NIA, and those infected hauled out and cleaned. Recommended treatment of vessels is to air dry for a minimum of 48 h and then manually remove NIA colonies so that fragments do not re-enter the water column. We recommend that vessel owners be required to present a certificate of cleanliness before being permitted to leave a harbour that is known to contain NIA.

7.5 CONTROL

If NIA cannot be prevented from entering a Newfoundland harbour or aquaculture site, or eradicated, control of further growth and spread is the most viable option. In fact, since prevention by regulating shipping is politically and practically intractable, and eradication is unlikely, control of growth and spread is likely the most feasible objective.

Since the recommended measures in Section 7.4 above (Eradication) are more likely to control population growth rate at a site than to eradicate NIA completely, we recommend all of them for control as well. In fact, whether eradication or control is the objective may simply be a matter of the frequency of application of the measures in Section 7.4. Eradication may require more frequent and sustained application of extermination measures, while control may require less frequent or sustained application.

Since colonial ascidians are most likely spread from site-to-site by fouled vessel hulls, we return to the primary importance of maintaining the coastal fleet free of fouling by NIA. In addition, since NIA are known to foul mussels, lobsters, and other shellfish, we recommend restriction of the movement of aquaculture equipment, aquaculture product, and etc., from colonized to un-colonized areas. If restriction of movement is not possible, we recommend cleaning of vessel hulls, aquaculture equipment, and etc., before movement from colonized to un-colonized areas. This would apply at any time of the year. Cleaning can be done by any effective method, but making sure that by-products of the process are not released back into the ocean.

The interprovincial vessel trade requires special mention. Buyers on the south coast of Newfoundland frequently purchase vessels in Nova Scotia and PEI, two provinces with many harbours containing *Botryllus schlosseri, Botrylloides violaceus*, and other NIA species (Sephton et al. 2011). One of the most heavily fouled vessels in Belleoram harbour was purchased in Nova Scotia (vessel owner, pers. comm.). We recommend immediate regulations regarding hull cleaning of vessels sailing between Nova Scotia, PEI, and Newfoundland. This may be the most important measure that can be put in place to control the invasion of Newfoundland harbours by NIA (Fig. 9).

7.6 MINIMIZE RISKS OF UNINTENDED CONSEQUENCES

Most of the techniques for eradication of NIA result in the complete extermination of all living organisms in the target area. Such a radical treatment can have many unintended

consequences. Thus, all methods must be applied in a way that minimizes unintended effects. In addition, parties carrying out the treatment must make sure they do not have legal liability for unintended outcomes.

Potential unintended effects of some of the recommended methods above are the opening of new space for NIA recruitment, the introduction of brooded larvae and colony fragments into the ocean increasing future recruitment, the production of near-bottom hypoxia or anoxia from decaying organisms, the destruction of benthic habitat important for valued species, the mortality or decreased yield of commercially important species, and the production of foul smelling gases offensive to humans. Biocides and introduced predators have many potential unintended consequences, and should be used cautiously, guided by the most recent research. Most importantly, we strongly recommend thorough thinking about unintended consequences before applying any eradication program.

7.7 FUTURE RESEARCH

7.7.1 Sediment suffocation and air bubbles

Ascidians collect food particles by filtering sea water through a fine mucous filter. This filter can become clogged if levels of suspended sediment are high. If clogged, the ascidians will starve and die. Research is needed to establish lethal sediment concentrations and exposure times for *Botryllus schlosseri*, *Botrylloides violaceus*, and *Ciona intestinalis*. Research is also needed on cost effective means for creating high concentrations of suspended sediments. Streams of air bubbles have been proposed as a possible mechanism to generate high concentrations of suspended sediment. In addition, bubbles themselves have been shown to scour surfaces clear of fouling organisms (Bullard et al. 2010). Bubble streams could conceivably be applied deep within the substructure of fixed wharves where colonial NIA may be immune to other eradication treatments. We recommend that research is carried out on the effect of bubble streams on the settlement and survival of colonial NIA in Newfoundland harbours.

7.7.2 Ultrasound

Small ultrasound generators are currently available for de-fouling vessel hulls (e.g., http:// www.ultrasonic-antifouling.com/powerboat_systems.html). These generators have apparently been successful in preventing biofouling of steel and fiberglass vessels. The use of these generators to prevent biofouling by NIA has not yet been tested. Furthermore, they have not been tested on wooden vessels or wharf pilings. In the case of wooden fouled surfaces, perhaps some form of projected ultrasound device may be effective. Trials of projected ultrasound conducted in PEI have been effective in killing NIA. There also has been an indication that hull-mounted ultrasound generators may prevent biofouling of nearby wharf pilings when vessels are tied up for a period of days or weeks. A major advantage of ultrasound is that it could be used year round. We recommend that research is conducted to determine the effectiveness of hull-mounted or projected ultrasound generators against NIA in Newfoundland coastal waters. Work will also be needed on the potential risk of ultrasound to surrounding native species.

7.7.3 Hull Cleaning

Many factors may affect the success of manual cleaning of vessel hulls. We recommend research on the optimal protocol, e.g. is it best to remove the NIA when wet, followed by drying and disposal, or allowing the material to dry for a period of days before removal and disposal? In addition, what is the best method for containing the waste material as it is accumulating, so as to prohibit introduction of any of the material back into the ocean?

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